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# Vitamins, microelements and the immune system: current standpoint in the fight against coronavirus disease 2019

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## Abstract

Coronavirus disease 2019 (COVID-19) is an acute respiratory disease associated with severe systemic inflammation. The optimal status of vitamins and microelements is considered crucial for the proper functioning of the immune system and necessary for successful recovery. Most patients with respiratory distress in COVID-19 are vitamin and microelement deficient, with vitamin D and Se deficiency being the most common. Anyway, various micronutrient supplements are widely and arbitrarily used for prevention or in the treatment of COVID-19. We aimed to summarise current knowledge about molecular and physiological mechanisms of vitamins (D, A, C, $B_6$, $B_9$ and $B_{12}$) and microelements (Se, Zn, Cu and Fe) involved in the immune system regulation in consideration with COVID-19 pathogenesis, as well as recent findings related to their usage and effects in the prevention and treatment of COVID-19. In the early course of the pandemic, several, mainly observational, studies reported an association of some micronutrients, such as vitamin C, D and Zn, with severity reduction and survival improvement. Still, emerging randomised controlled trials showed no effect of vitamin D on hospitalisation length and no effect of vitamin C and Zn on symptom reduction. Up to date, there is evidence neither for nor against the use of micronutrients in the treatment of COVID-19. The doses that exceed the recommended for the general population and age group should not be used, except in clinical trials. Benefits of supplementation are primarily expected in populations prone to micronutrient deficiencies, who are, as well, at a higher risk of worse outcomes in COVID-19.

**Key words:** **Micronutrients: Vitamins: Microelements: Immune system: Supplementation: COVID-19**

Coronavirus disease 2019 (COVID-19) is an acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the WHO declared the pandemic in March 2020, more than 230 million infections and around 4·8 million deaths have been reported around the globe[1]. Pending the distribution of newly developed vaccines, the health care system is still facing unprecedented pressure. Apart from being an acute respiratory disease, COVID-19 is associated with broad-spectrum symptoms of systemic inflammation and multiple organ dysfunction that leads to the persistence of symptoms and long-term complications[2–4]. COVID-19 is characterised by immunosuppression in the first stage (or non-severe symptomatic period) and hyper-inflammation in the second stage (or severe symptomatic period)[5,6]. A well-coordinated immune response promotes recovery, while an uncontrolled systemic inflammatory response leads to complications and death[5]. SARS-CoV-2 infection activates a strong innate immune response in epithelial cells and alveolar macrophages followed by neutrophil/monocyte infiltration[5]. The SARS-CoV-2 virus appears to strongly activate respiratory burst in neutrophils, generation of reactive oxygen spaces and formation of neutrophils extracellular traps (NET)[7,8]. The process of NETosis may contribute to microthrombotic events that drive organ damage in COVID-19[7]. Additionally, exhaustion and decrease in the number of lymphocytes, particularly T regulatory lymphocytes (Treg), are found in patients with severe COVID-19 and might add to the loss of regulatory functions and cytokine storm[9,10]. The release of cytokines such as TNF $\alpha$, IL-1$\beta$ and IL-6 disrupts alveolar-capillary barrier integrity and results is in acute respiratory distress syndrome (ARDS), extensive microthrombus formation and multiple organ failure[5,7].

**Abbreviations:** ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; NET, neutrophils extracellular traps; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VDR, vitamin D receptor.

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The optimal status of specific nutrients is considered of uttermost importance for the proper functioning of the immune system. The European Food Safety Authority declared six vitamins (D, A, C, folate, $B_6$ and $B_{12}$) and four microelements (Zn, Fe, Cu and Se) as essential for the normal functioning of the immune system[11]. A recent study in COVID-19 patients showed that most of the patients with respiratory distress were vitamin deficient, whereas vitamin D and Se deficiency were the most commonly found[12]. In that respect, the effect of supplementation with vitamins and microelements has been studied in various registered clinical trials up to date, sometimes with conflicting results. Besides, supplements have been commonly used as prophylaxis in healthy, or as adjunctive treatment in COVID-19 patients, even without medical prescription.

We aimed to summarise and discuss current evidence-based knowledge about the physiological role of vitamins and microelements in the immune system regulation in consideration with COVID-19 pathogenesis, as well as recent findings related to their usage and potential effects in the treatment of COVID-19 patients.

## Methods

PubMed database was searched for articles on vitamins and microelements, their role in the immune system or the prevention or treatment COVID-19. Specifically, the following search terms were used: (1) COVID-19 OR SARS-CoV-2 OR CoronaVirus AND (Vitamin D OR Vitamin A OR Vitamin C OR Vitamin $B_6$ OR Folate OR Vitamin $B_{12}$ OR Selenium OR Zinc OR Copper OR Iron). This part of the search included papers published from December 2019 to May 2021. In addition, we used the following search terms to explore potential mechanisms of vitamins and microelements involved in the immune system regulation: (2) Immune system OR Immune response AND (Vitamin D OR Vitamin A OR Vitamin C OR Vitamin $B_6$ OR Folate OR Vitamin $B_{12}$ OR Selenium OR Zinc OR Copper OR Iron). This part of the search included papers published from January 2000 to May 2021. The reference lists of relevant articles were also reviewed to identify additional appropriate articles. The abstracts were evaluated by two independent pairs of reviewers (B. Djordjevic and A. Velickov; J. Milenkovic and D. Stojanovic), and papers considered not relevant were excluded. The selected studies were evaluated in full text to identify molecular and physiological mechanisms of vitamin/micronutrient actions in consideration with COVID-19 pathogenesis or crucial interventions and outcomes related to the use of vitamins/micronutrients in the prevention/treatment of COVID-19.

## *Vitamins against coronavirus disease 2019*

***Vitamin D.*** Vitamin D is a group of fat-soluble secosteroids that caught a lot of attention due to the potential role in the prevention and treatment of COVID-19. Beyond its well-known function in Ca homoeostasis, vitamin $D_3$ exerts a profound effect on the immune responses, both innate and adaptive. It might have led to prompt empirical inclusion of vitamin D as an adjunctive treatment in COVID-19. However, current guidelines and several studies argue that there is still not enough evidence to support its use in the prevention and treatment of the SARS-CoV-19 infection. There is a need for well-designed RCT; hence, further investigations are encouraged[13–16].

***Mechanisms of vitamin D immunomodulatory actions.*** Generally, after its synthesis or ingestion, vitamin $D_3$ is metabolised into 25-hydroxyvitamin D3 (25(OH)D) in the liver. Circulating 25(OH)D is then activated by the 1$\alpha$-hydroxylase (CYP27B1) into the 1$\alpha$,25-dihihydroxyvitamin D3 (1,25(OH)2D), which exerts autocrine and paracrine functions[17,18]. The major circulating form is 25(OH)D, and its serum concentrations are measured to assess vitamin D status. A total 25(OH)D cut-off serum levels of $\geq$ 30 ng/ml (75 nmol/l) are generally considered sufficient, the levels of 20–29 ng/ml represent vitamin D insufficiency, and below 20 ng/ml (50 nmol/l) a deficiency[16,19,20].

The rationale for the wide use of vitamin D in COVID-19 patients is based on its immunomodulatory and anti-inflammatory effects, which might prevent cytokine storm syndrome and severe lung damage. Many vitamin D beneficial effects on immune functions have been described so far[15,17,21–23]. It induces potent antimicrobial effects in human monocytes that are dependent on its active form and vitamin D receptor (VDR). Following Toll-like receptor (TLR)1/2 heterodimer activation, the VDR gene is upregulated in monocytes and macrophages, and the CYP27B1 gene in dendritic cells. Therefore, there is local activation of 25(OH)D, which further upregulates defensins and microbicidal peptides such as cathelicidin LL-37 and CYP24-hydroxylase (vitamin D-inactivating enzyme)[18]. Besides being capable of attacking intracellular microbes, cathelicidin acts against fungi and invading respiratory viruses[17]. Additionally, vitamin D impairs TLR9-induced IL-6 production in human peripheral blood monocytes, whereas TLR3 (endosomal dsRNA detector) remains unaffected[24]. T-cell cytokines may influence innate immune responses by modulation of vitamin D metabolism. Interferon $\gamma$ (IFN-$\gamma$) upregulates CYP27B1, enhancing the conversion of 25(OH)D, while IL-4 induces CYP24 hydroxylase[22].

Vitamin D increases IκB$\alpha$ mRNA and protein levels, while its receptor, VDR, decreases NF-κB activity by physical interaction with IκB kinase $\beta$[25]. 1,25(OH)2D3 may further increase the inhibitory activity of VDR. By blocking of NF-κB pathway, vitamin D downregulates many cytokine genes, including genes for plasminogen activator inhibitor1, renin and angiotensinogen[25]. Specifically, it was determined to inhibit the TNF family of genes, IFN-$\gamma$, IL-2, IL-6, IL-12, IL-17 and IL-22[15,20,21,26–28], while increasing the anti-inflammatory IL-10 involved in producing Tregs[26].

Many additional potentially beneficial effects of vitamin D against COVID-19 are being proposed, such as altered ACE2 receptor expression, inhibition of renin action and angiotensin II (Ang II) accumulation, maintenance of epithelial gap junctions, protective effect on the alveolar epithelium (trophic and anti-apoptotic effect) and anti-thrombotic actions[16,29,30]. In an animal model, vitamin D attenuated acute lung injury, by the inhibition of angiopoietin-2-Tie-2 signalling, thus creating protection of vascular barrier. Vitamin D receptor is highly expressed in the lungs, and VDR-null mice exhibited more severe lung injury, with neutrophil infiltration, pulmonary

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inflammation, vascular leakage and increased pulmonary levels of renin and Ang II[29].

*The use of vitamin D in prophylaxis and therapy of coronavirus disease 2019.* Two recent meta-analyses found that vitamin D supplementation is safe and effective in preventing acute respiratory infections overall, especially in those with vitamin D deficiency at baseline (< 25 nmol/l) and when taken in recommended doses for a longer period[31,32].

Unfortunately, the elderly with chronic diseases and patients with ventilator-acquired pneumonia are likely to have vitamin D deficiency[27,28]. Vitamin D deficiency was also reported to be common in people who develop ARDS, especially in those who died[30]. The population at higher risk for COVID-19 has the same risks as for vitamin D deficiency, such as older age, African Americans, obese, cancer patients, chronic kidney failure or liver disease, autoimmune conditions, pregnant females, healthcare workers, etc[13,17–19].

A substantial number of observational small-scale studies and systematic reviews reported the important role of vitamin D in decreasing the risk for COVID-19 and related mortality, supporting its prophylactic and therapeutic use[14,16,17,20,33–37]. A recent study investigated 25(OH)D status in 287 adult COVID-19 patients and revealed an independent association between a total of 25(OH)D levels of ≥ 30 ng/dl and decreased risk of mortality in elderly and non-obese COVID-19 patients[17]. Similarly, a study that correlated data of COVID-19 cases and deaths per 1 million general population (Europeans) to the vitamin D serum levels found a significant negative correlation between mean vitamin D concentrations and an incidence of COVID-19 cases, but not deaths[16]. The meta-analysis by Kazemi *et al.*[38] indicated a significant relationship between vitamin D status and SARS-CoV-2 infection, COVID-19 composite severity and mortality, simultaneously emphasising the study limitations reflected in heterogeneity in methodological and statistical approaches.

Since a substantial number of people do not get enough vitamin D from natural sources, there is a question of whether it is possible to increase vitamin D levels quickly enough, with high bolus doses, after one gets COVID-19. Is it going to be effective and safe? Besides, the vitamin D serum threshold that would provide some protection against COVID-19 is not determined[28].

National Institute for Health and Care Excellence recommends vitamin D supplementation of 10 μg (400 μg) a day, which would be enough to prevent serum 25(OH)D from falling below 10 ng/dl (< 25 nmol/l). The tolerable UL for adults is 100 μg (4000 μg) a day and should not be exceeded[13]. USA National Academy of Medicine and the European Food Safety Authority supports a target blood level of vitamin D of at least 20 ng/ml (50 nmol/l), which requires supplementation of 800 μg a day[39]. The Endocrine Society's Clinical Guidelines suggest that raising 25(OH)D levels above 30 ng/ml may require at least 1500–2000 μg a day, while the maintenance tolerable UL should not exceed 4000 μg a day[19].

However, people who become SARS-CoV-2 infected might need higher vitamin D doses, especially those already deficient[20,23]. There are several recommendations regarding vitamin D supplementation, but the goal should be to raise 25(OH)D levels above 40 ng/ml (100 nmol/l)[13,19,20,39]. According to Heaney *et al.*[40], when starting from 25(OH)D baseline level of around 20 ng/ml (70 nmol/l), it takes about a month to reach 60 ng/ml with 10 000 μg a day and about three months with 4000 μg a day. According to the Endocrine Society Clinical Practice Guidelines, adults who are vitamin D deficient should be treated with 50 000 μg once a week for 8 weeks or 6000 μg a day to achieve a level of 25(OH)D above 30 ng/ml, followed by the maintenance therapy of 1500–2000 μg/d[19].

There are no clear results on whether short-term high doses of vitamin D might improve outcomes in critically ill patients[37,41,42]. In the VITdAL-ICU study, vitamin D was given enterally once at a dose of 540 000 μg, followed by monthly maintenance doses of 90 000 μg, to critically ill patients with vitamin D deficiency. There was no general reduction in hospital length of stay nor mortality, except for in-hospital mortality of those who were severely vitamin D deficient (≤ 12 ng/ml)[41]. Similarly, a single dose of 200 000 μg of vitamin D did not affect hospital length of stay in patients with moderate to severe COVID-19[43]. In contrast, vitamin D booster therapy in a dose of 280 000 μg in a period of up to 7 weeks appears to be associated with a reduced risk of mortality in COVID-19 patients[37]. In another study, vitamin D supplementation gave significant protection against respiratory infections in those receiving daily or weekly vitamin D doses, but not in those with one or more bolus doses (of at least 30 000 μg)[14].

Prolonged use of bolus doses could cause wide fluctuations in 25(OH)D circulating levels, which might provoke dysregulation of vitamin D hydroxylases, with possible cellular maladaptation and impairment of respiratory epithelium[44]. Another concern is that high doses in a short period, which would improve serum levels may not provide and sustain overall body needs. Therefore, it might be preferable to gradually achieve sufficient and stable 25(OH)D serum levels.

When vitamin D is used in COVID-19 treatment, caution should be taken regarding adverse effects, especially hypercalcemia, and related organ dysfunctions[13,15,19,20].

It is worth mentioning that critically ill COVID-19 patients might benefit from combined vitamin D and anti-IL-6 (tocilizumab) treatment, proposed in severe forms of pneumonia[45]. A single shot of vitamin D (300 000 μg) was determined to diminish increased serum IL-6 levels in previously vitamin D-deficient patients with ventilator-acquired pneumonia and was associated with reduced mortality[27]. Besides, a better therapy response to tocilizumab was achieved after 6 months in those rheumatoid arthritis patients that had sufficient serum vitamin D levels (≥ 30 ng/dl)[21].

Taken together, since vitamin D supplementation is determined effective against acute respiratory tract infections, people with high risk for severe COVID-19 should take vitamin D to maintain their serum 25(OH)D levels in the optimal range (> 30–40 ng/dl). Daily or weekly intake of recommended tolerable doses, without bolus doses, was showed protective and safe[14,20]. Treatment that includes high bolus doses of vitamin D needs to be further investigated for efficacy and safety.

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*Implications for the use of vitamin D in patients that require mechanical ventilation or ICU admission.* Given the suggestions for vitamin D intake in COVID-19 patients, one should consider concomitant adjustments of phosphate and Mg levels, the elements that are usually overlooked. However, they are in the middle of the complex interactions between vitamin D, hormones, acid–base balance and hypoxia. Since severe phosphate and/or Mg deficiencies lead to energy shortage and metabolic abnormalities, they might contribute to the disease progression[46–48]. SARS-CoV-2 caused acute hyperinflammatory response leads to cellular ATP depletion and immune cell dysfunction, while phosphate and Mg are required for ATP regeneration[48]. Several studies have already shown a significant correlation between phosphate deficiency and COVID-19 severity. Non-survivors had lower serum phosphate levels than survivors, which correlated to lymphocyte counts and severity of lung damage[46,47,49].

The risks for severe COVID-19 are the same as for hypophosphatemia and hypomagnesemia, e. g. older age, obesity, diabetes, renal or liver dysfunction[46,50]. Additional risks may emerge during the disease course, such as diminished food intake, diarrhoea, renal dysfunction, diuretics, hyperglycaemia or insulin use, antacids, corticosteroids or inadequate mechanical ventilation. Hyperventilation can easily aggravate hypophosphatemia due to respiratory alkalosis, which stimulates ATP production and consumes serum phosphates[46,51].

Symptoms of hypophosphatemia and hypomagnesemia are in line with the COVID-19 manifestations, the most common being a general weakness. Others include myopathy and cardiomyopathy, thrombocytopenia and increased platelet aggregation, coagulopathy, neurologic disturbances (encephalopathy, confusion, delirium, paresthesia and dysarthria), immunodeficiency, disturbed tubular transport, dysregulated vitamin D metabolism and failure-to-wean from mechanical ventilation. Importantly, hypophosphatemia impedes 2,3-bisphosphoglycerate in erythrocytes, which regulates haemoglobin affinity toward oxygen, and so may aggravate hypoxia[46,48,50,51].

## Vitamin A

Vitamin A or retinol represents a group of unsaturated monohydric alcohols that contain a beta-ionone ring to which an isoprenoid chain is attached. Foods of animal origin contain a high concentration of retinol and retinyl-esters, while fruits and vegetables are rich in provitamin $\beta$-carotene. RDA for vitamin A is 900 µg for adult males and 700 µg for females, but is higher in pregnant and lactating individuals. Deficiency is uncommon in high-income countries, but is found in vulnerable groups such as infants, children, pregnant and lactating women in low-income countries[52,53].

Retinol is the precursor to two active metabolites: retinal, essential for the vision process, and retinoic acid, which acts as an intracellular messenger that affects gene transcription by binding nuclear retinoid receptors (RAR and RXR). The RAR-RXR heterodimer binds retinoic acid response elements that are typically found in gene promoters or enhancers regions of the DNA[54]. Similarly, the VDR forms heterodimers with the retinoid × receptor (RXR). RAR-RXR and VDR-RXR heterodimers, as ligand-dependent transcription factors, bind particular regions of DNA that are responsive to vitamins A and D, respectively. Since both vitamins A and D may compete for binding to RXR and DNA, they might act as antagonists[55].

*Mechanisms of vitamin A anti-inflammatory actions.* Apart from maintenance of vision, vitamin A regulates growth, cell differentiation, embryonic and fetal development, epithelial barrier function and immunity[56]. Vitamin A is involved in the regulation of both innate and adaptive immune responses. Alveolar macrophages are critical to the homoeostasis of the inflammatory environment in the lung and typically exhibit hybrid M1/M2 phenotype, thus maintaining an equilibrium between immune tolerance and protective immunity in the alveolar space[57]. While infections might induce monocyte differentiation towards the M1 lineage, vitamin A promotes an M1 to M2 phenotype shift, thus inhibiting macrophages-mediated pro-inflammatory reaction[58]. Additionally, by releasing cytokines, macrophages may influence the degree of activity or maturation of neighbouring dendritic cells and thus influence antigen presentation in lungs[59]. Dendritic cells not only initiate the innate immune response but also present antigens to naive T cells, thus activating adaptive immunity[60]. Dendritic cells express enzymes in the vitamin A metabolic pathway that is capable of conversion of retinol into its active form, which, after being released from the cell, activates macrophage-mediated antimicrobial responses[61]. Vitamin A promotes T cell migration towards the area of inflammation and T cell differentiation and poly-clonal response in a dose-dependent manner[62]. Besides, it mediates in TGF-ß-dependent conversion of T cells into T regulatory (Treg) cells, thus preventing autoimmunity. However, in the presence of IL-6 and TGF-$\beta$ may act towards autoimmunity and inflammation by inducing T helper 17 response[62].

Retinoic acid-inducible gene I-like receptors represent a direct link between vitamin A and viral diseases. Retinoic acid-inducible gene receptors recognise cytosolic viral RNA and mediate the transcription of type I interferon involved in antiviral host response[63]. Coronaviruses including SARS-CoV-2 may suppress type I interferon antiviral responses and cause disturbance of the delicate balance between immune-suppressive Tregs and pro-inflammatory T helper 17 cells[63,64]. Additionally, active forms of vitamin A have a direct effect on IgA plasma cell differentiation which affects the synthesis and secretion of IgA[65].

*The use of vitamin A in the therapy of infectious diseases and implications for the use in coronavirus disease 2019.* Up to date, there is no consistent evidence for the beneficial effects of vitamin A supplementation in adults and the elderly in the treatment of infections[66]. In children, positive effects in preventing lower respiratory tract infections appear to be limited to populations with acute and chronic undernutrition[67]. Vitamin A supplementation, along with vaccination, improves survival after challenge with a high dose of pneumococcus in animals and clinical trials in children have been initiated (clincaltrials.gov, PCVIT NCT03859687)[54].

Although, in theory, vitamin A supplementation might be beneficial in COVID-19, especially in the second inflammatory phase of the disease, up to date there are no data regarding its

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use in patients and only a few RCT have been registered[68]. The data from the clinical trials regarding the prevention of acute lower respiratory tract infections in children certainly advise approach with caution, especially in high-income countries where vitamin A deficiency is rare[66].

### Vitamin C

Vitamin C or L-ascorbic acid is a water-soluble vitamin with a chemical structure similar to carbohydrates that cannot be synthesised in humans due to the lack of an enzyme l-gulono-γ-lactone oxidase. RDA for vitamin C has been set to 90 mg/day for males and 75 mg/d for females, whereas smokers require 35 mg/d more. Adverse effects related to vitamin C supplementation are observed when daily intake is higher than 3 g and include diarrhoea and other gastrointestinal disturbances, increased oxalate and uric acid excretion, changes in Fe, Cu and $B_{12}$ absorption and erosion of dental enamel[69]. In balanced diets, most of the daily intake of vitamin C comes from fruits and vegetables. Due to the transporter-mediated absorption and saturation phenomenon, the bioavailability of vitamin C after oral intake decreases with the increase of the oral dose in the range from 200 to 1000 mg, which can be bypassed by intravenous administration[70].

*The role of vitamin C in immune system regulation implicated in coronavirus disease 2019.* Vitamin C is a highly effective antioxidant and a co-factor in the process of collagen and neurotransmitter/hormone synthesis. Therefore, it is essential for the barrier function of skin/mucosa, blood vessel integrity and cardiovascular response to severe infection[71,72]. It contributes to the proper function of both the innate and adaptive immune systems. Vitamin C accumulates in neutrophils and enhances chemotaxis, phagocytosis and apoptosis of the neutrophils, thereby decreasing NETosis and excessive tissue damage[72]. Additionally, it accumulates in lymphocytes and affects lymphocyte proliferation, including Treg cells. The effect on the immune cells, especially the decrease in NETosis, could be beneficial for COVID19 patients since SARS-CoV-2 can stimulate extracellular neutrophils traps (NET) and activate NETosis in neutrophils that contributes to multiorgan failure[7].

*Vitamin C in the therapy of coronavirus disease 2019.* Although it was previously shown that oral administration of vitamin C in doses of 1–3 g/d reduces the length of ICU stay, hospital stay and the duration of mechanical ventilation in ICU patients[73,74], the benefits of intravenous/oral high dose administration of Vitamin C in ARDS and COVID-19 patients was not shown up to date in RCT[75–77]. No significant benefit was reported in the reduction of symptoms, hospitalisations, SpO2 at discharge, the length of ICU stay and mortality in COVID-19 patients who were administered Vitamin C in high doses[76,77]. Besides, high doses of Vitamin C did not significantly improve organ dysfunction scores, inflammation marker levels and vascular injury in sepsis and ARDS[75]. However, ambulatory treated COVID19 patients who received 8 g/d of vitamin C reported adverse effects such as nausea, diarrhoea and stomach cramps in a higher proportion than non-treated individuals[77].

The available data suggest that there is no benefit in supplementing vitamin C in high doses in the treatment of COVID-19 patients. However, high doses of vitamin C have been associated with pro-oxidative effects especially through the interaction with transition metal ions such as Fe and Cu[78]. Since Fe overload has been implicated in the pathogenesis of COVID-19[79], further research regarding the use of vitamin C might be needed, especially evaluation of lower doses and therapy duration.

### Vitamin B

*Folate (Vitamin $B_9$).* Folate or $B_9$ is a naturally occurring water-soluble B vitamin that consists of pteridine ring, para-aminobenzoic acid and glutamate residues. RDA for folate is 400 μg for both adult males and females; however, pregnant women require 600 μg of folate daily. Although the deficiency is rare due to the food fortification program, supplementation is advised to women of childbearing age, especially during pregnancy and lactation[52,80].

*The role of folate in immune system regulation.* Folate acts as a coenzyme or co-substrate in methylation reactions for nucleic acids and protein synthesis and amino acids metabolism. Additionally, folate is required for ATP synthesis and the proper function of the immune system. Purine molecules, inosine and adenosine modulate the proliferation and cytotoxic activity of NK cells in response to pathogens, as well as adaptive responses by secreting cytokines such as IL-1β and IFN-γ[81]. Folate is a survival factor for regulatory T (Treg) cells that express high levels of folate receptor 4[82].

*Implications for the use of folate in the therapy of coronavirus disease 2019.* Based on molecular docking studies, two recent pre-prints hypothesised that folic acid might be beneficial in the early stages of COVID-19 due to the potential inhibition of furin, which enables entry to the host cells or 3CLpro (Mpro) protease of SARS-CoV-2 virus[83,84]. However, SARS-CoV-2 requires host folate and one-carbon metabolism to support nucleotide synthesis and viral replication, which can be inhibited by folate inhibitors such as methotrexate[85]. Although low serum folate levels are present among hospitalised patients with COVID-19, there is no association between serum folate levels and incidence of hypoxemia, invasive ventilation, length of hospital stay and mortality[86]. At this moment, further studies are needed to assess the potential benefit of folate supplementation in COVID-19 patients, especially regarding the evidence that suggests that folic acid supplementation might reduce the hospitalisation rate in pregnant women with SARS-CoV-2 infection[87].

*Vitamin $B_6$.* Vitamin $B_6$ is a water-soluble vitamin whose active form pyridoxal 5′-phosphate acts as a coenzyme in various enzymatic reactions such as synthesis and/or degradation of amino acids, neurotransmitters, sphingolipids, haemoglobin and glycogen. RDA for vitamin $B_6$ is 1·3 mg/d, but is higher in pregnancy, lactation and the elderly. Deficiency is uncommon, but lower plasma levels are commonly found in individuals with renal function impairment, autoimmune disease and alcoholics[52,88].

**Table 1.** Vitamins and microelements and their roles and effects related to the immune system

| Vitamin/microelement | Immunomodulating and other defense effects related to COVID-19. | | | Refs. |
|---|---|---|---|---|
| | Innate immunity | Adaptive immunity | Other, immunity related | |
| Vitamin D | Induces potent antimicrobial effects in monocytes.<br>Upregulates defensins and microbicidal peptides (cathelicidin).<br>Stimulates high endogenous TGF-$\beta$1 expression. | Blocks the canonical NF-κB activation pathway.<br>Hinders development of Th17, Th9 and Th1 cells.<br>Supports differentiation of Th2 and Tregs.<br>Inhibits TNF family, IFN-$\gamma$, IL-2, IL-6, IL-12, IL-17, IL-22, etc.<br>Increases anti-inflammatory IL-10. | Alters ACE2 receptor expression.<br>Inhibits renin and angiotensin II actions.<br>Maintains epithelial gap junctions.<br>Protects alveolar epithelium (trophic and anti-apoptotic effects).<br>Anti-thrombotic actions, etc. | (15–18,20–29) |
| Vitamin A | Promotes a M1 to M2 phenotype shift. | Promotes T cell migration and differentiation.<br>Promotes polyclonal response.<br>Mediates TGF-$\beta$-dependent conversion of T cells into Tregs.<br>Direct effect on IgA plasma cell differentiation. | Epithelial barrier function.<br>Promotes mucin secretion.<br>Regulates growth and cell differentiation. | (56,58,62,65) |
| Vitamin C | Enhances neutrophils chemotaxis, phagocytosis and apoptosis.<br>Decreases NETosis. | Affects lymphocyte proliferation including Treg cells. | Highly effective antioxidant.<br>Essential for barrier function of skin/mucosa.<br>Maintains blood vessels integrity.<br>Co-factor in collagen and neurotransmitter/hormone synthesis. | (71–72) |
| Vitamin $B_6$ (pyridoxal) | Inhibits NLRP3 inflammasome activation thus prevents IL-1$\beta$ synthesis. | via S1P metabolism it regulates lymphocyte trafficking.<br>Involved in the regulation of cytokine synthesis.<br>Inhibits IL-6-positive feedback cycle. | Involved in regulation of endothelial barrier permeability.<br>Normalises homocysteine levels – regulates NO.<br>Cofactor in various enzymatic reactions. | (89–93, 99, 101) |
| Vitamin $B_9$ (folate) | Modulates the proliferation and cytotoxic activity of NK cells. | Survival factor for Tregs. | Required for ATP synthesis.<br>Normalises homocysteine levels – regulates NO. | (81–82, 99, 101) |
| Vitamin $B_{12}$ (cobalamine) | Necessary for leukocyte proliferation.<br>Supports NK cell activity. | Supports CD8 + T cell proliferation and activity. | Important role in methylation, DNA, protein and lipid synthesis.<br>Normalizes homocysteine levels – regulates NO. | (96, 99, 101) |
| Selenium | Influences leukocyte interactions, TLR signalling and microbicidal processes.<br>Involved in switch of M1 towards M2 phenotype.<br>May indirectly enhance NK and cytotoxic T-cell activities.<br>Influences expression of inflammatory mediators. | Established inhibitor of NF-kB.<br>Skews T cells towards Th1 phenotypes.<br>Helps Tregs differentiation.<br>Stimulates lymphocyte proliferation to a mitogen *in vitro*.<br>Improves activation and proliferation of B-lymphocytes.<br>Induces stronger response to some vaccines (polio). | Potent extracellular antioxidant.<br>Influences NO production.<br>Regenerates ascorbic acid.<br>Involved in post-translational protein modifications.<br>Reduces thromboxane A2 fomation and platelet aggregation.<br>Insulin synthesis and signalling.<br>Thyroid function. | (69, 105, 111) |
| Zinc | Inhibits NETosis and degranulation of neutrophils.<br>Reduces neutrophil infiltration in the lungs during sepsis. | Influences cytokine production and release. | Catalyses various enzymes activity.<br>Contributes to protein structure and gene expression.<br>Maintains the membrane barrier structure and function.<br>Increased cell conc. impairs replication of RNA viruses. | (129, 131–133, 135) |
| Copper | Potentiates respiratory burst and antimicrobial effects.<br>Involved in neutrophil and macrophage functions. | Involved in cytokine synthesis and T cell proliferation. | Influences iron and zinc metabolism. | (148–150) |

ACE2, angiotensin-converting enzyme 2; TGF-$\beta$1, transforming growth factor $\beta$1; M1, pro-inflammatory macrophage phenotype; M2, anti-inflammatory macrophage phenotype; Treg, T regulatory cells; S1P, sphingosine 1-phosphate; NET, neutrophil extracellular traps.

**Table 2.** Registered interventional RCT of vitamins/microelements supplementation in COVID-19 that were completed by September 2021 (clinicaltrials.gov; covid-19.cochrane.org)

| Number of registered RCT | Title | Status | Intervention | Primary outcomes |
|---|---|---|---|---|
| Interventional studies completed by September, 2021 | | | | |
| NCT04449718 | Vitamin D Supplementation in Patients With COVID-19: A Randomized, Double-blind, Placebo-controlled Trial | Completed | Vitamin D: single dose 200 000 μg on admission | • Length of hospitalisation[43] |
| NCT04793243 | Vitamin D3 Levels in COVID-19 Outpatients From Western Mexico: Clinical Correlation and Effect of Its Supplementation | Completed | Vitamin D: 10 000 μg/daily | • Effects of supplementation on serum levels of 25(OH)D and prognostic markers in COVID-19 patients |
| NCT04407286 | Vitamin D Testing and Treatment for Adults With COVID-19 | Completed | Vitamin D: 10 000 μg/day (age 18–69 years) or 15 000 μg/day (age 70+) | • Vitamin D levels and severity of COVID-19 symptoms |
| IRCT20200411047025N1 | Evaluation of effectiveness of Intravenous vitamin c in Patients with COVID-19 Referred to Imam Khomeini Hospital: a clinical trial | Completed | Vitamin C:1500 mg in slow infusion (1–2 h) with normal saline. | • Improvement of SPO2 (peripheral capillary oxygen saturation)[76] |
| NCT04342728 | Coronavirus Disease 2019- Using Ascorbic Acid and Zinc Supplementation (COVIDAtoZ) Research Study A Randomized, Open Label Single Center Study | Completed | Zinc: 50 mg zinc gluconate or vitamin C: 8000 mg | • Symptom reduction[77] |
| NCT04357782 | Administration of Intravenous Vitamin C in Novel Coronavirus Infection and Decreased Oxygenation (AVoCaDO): A Phase I/II Safety, Tolerability and Efficacy Clinical Trial | Completed | Vitamin C:50 mg/kg in infusion given every 6 h for 4 days (16 total doses) | • Incidence of adverse events<br>• Incidence of serious adverse reactions<br>• Incidence of adverse reactions |
| NCT04733625 | The Effect of Vitamin D Therapy on Morbidity and Mortality in Patients With SARS-CoV 2 Infection | Completed | Vitamin D: 200 000 μg, single injection | • Death or need for intubation |
| NCT04446104 | A Randomized Open-label Prophylaxis Trial Among Migrant Workers at High-risk of COVID-19 (DORM Trial) | Completed | Zinc:80 mg/vitamin C: 500 mg daily for 42 days or vitamin C 500 mg daily for 42 days | • Laboratory-confirmed COVID-19[140] |
| NCT04344041 | COvid-19 and Vitamin D Supplementation: a Multicenter Randomized Controlled Trial of High Dose *v.* Standard Dose Vitamin $D_3$ in High-risk COVID-19 Patients (CoVitTrial) | Completed | Vitamin D: 400 000 μg or 50 000 μg in a single oral dose | • All-cause mortality |
| NCT04411446 | Randomized Controlled Trial of High Dose of Vitamin D as Compared With Placebo to Prevent Complications Among COVID-19 Patients | Completed | Vitamin D: 500 000 UI in a single oral dose | • Respiratory component of the sequential organ failure assessment score (SOFA score)<br>• Need of a high dose of oxygen or mechanical ventilation |
| NCT05037253 | The Effect of Vitamin D Supplementation in Reducing COVID-19 Morbidity Among Healthcare Workers | Completed | Vitamin D: high dose (50 000 μg weekly) on the first and second week, followed by a switch to a daily intake of 5000 μg for 3 months or low dose (2000 μg weekly) for 3 months | • Morbidity |
| NCT04883203 | The Effect of Vitamin D Supplementation on Recovery Delays for Non-Severe COVID-19 Cases | Completed | Vitamin D: 200 000 μg/1 mL | • Vitamin D supplementation and recovery delay in COVID-19 patients<br>• Delay between the first positive RT-PCR and the second negative RT-PCR |
| NCT04551339 | Zinc *v.* Multivitamin Micronutrient Supplementation to Support Immune Health in the Setting of COVID-19 Pandemic: A Randomized Study | Completed | Zinc: high dose in combination with copper, vitamin C/E and beta-carotene (PreserVision AREDS) formulation or multivitamin supplement with 11 mg of zinc (low dose) | • COVID-19 illness requiring hospitalisation |
| NCT04910230 | Efficiency and Safety of Nicotinamide-based Supportive Therapy in Lymphopenia for Patients With COVID-19: A Randomized Clinical Trial | Completed | Nicotinamide: 500 mg/daily in 5 doses, in addition to usual care | • The changes in absolute lymphocyte counts (*10^9/L) in before and 48 h after treatment |

*The roles of vitamin $B_6$ in the immune system regulation implicated in coronavirus disease 2019.* It has been proposed that vitamin $B_6$ supplementation improves immune functions in humans and animals, possibly by affecting metabolic pathways that result in metabolites with immunomodulating effects such as kynurenine and transsulfuration pathway and sphingosine 1-phosphate metabolism[89]. Sphingosine 1-phosphate metabolism regulates lymphocyte trafficking, endothelial barrier permeability and cytokine synthesis[90]. In $B_6$ deficiency, amplified kynurenine signalling through the aryl hydrocarbon receptor (Ahr) may stimulate an IL-6 positive feedback cycle, which could amplify inflammation[91,92]. Additionally, vitamin $B_6$ prevents IL-1$\beta$ synthesis by inhibiting NLRP3 inflammasome activation[93]. Therefore, it was recently proposed that vitamin $B_6$ might suppress hyperinflammation and cytokine storm syndrome in COVID-19, but this hypothesis should be tested in clinical trials[94].

*Vitamin $B_{12}$.* Vitamin $B_{12}$ (cobalamin) is a water-soluble vitamin with a complex molecular structure that contains a cobalt atom in the centre of the corrin ring bound to various side groups. The active forms of vitamin $B_{12}$ (hydroxo-, adenosyl- and methylcobalamin) play an important role in methylation, DNA, protein and lipid synthesis. RDA for vitamin $B_{12}$ is 2·4 μg, and this amount is commonly acquired through food. The absorption is dependent on the presence of intrinsic factor, and deficiency is found in pernicious anaemia, gastric resections and in some cases might be related to the use of certain medications[52,95].

*The roles of vitamin $B_{12}$ in the immune system regulation implicated in coronavirus disease 2019 and vitamin $B_{12}$ in the therapy of coronavirus disease 2019.* When it comes to the immune system, vitamin $B_{12}$ appears to affect lymphocyte count and the activity of NK cells. Vitamin $B_{12}$ deficiency leads to a decrease in absolute numbers of CD4+ and CD8+ lymphocytes, increases CD4/CD8 ratio and reduces the activity of NK cells[96]. Besides, the changes in a number of the lymphocytes and NK cells activity observed in vitamin $B_{12}$ deficiency can be reversed by vitamin $B_{12}$ replacement therapy[97]. Interestingly, COVID 19 is characterised by significant decreases in both CD4+ and CD8 + T cell counts[98]. Also, it was shown recently that vitamin $B_{12}$ supplementation in combination with vitamin D and Mg was associated with a significant reduction in the proportion of patients requiring oxygen support, intensive care support, or both, in older COVID-19 patients[33]. The promising results emphasise the need for further investigation to assess the ameliorating effect of vitamin $B_{12}$ on the severity of COVID-19.

*Folate/Vitamin $B_6$/Vitamin $B_{12}$ and hyperhomocysteinemia: implications in coronavirus disease 2019.* Plasma homocysteine levels are elevated in vitamin $B_{12}$ deficiency, vitamin $B_6$ deficiency and the folic acid deficiency[99]. Hyperhomocysteinemia leads to a reduction in endothelial nitric oxide bioavailability and induces inhibition of nitric oxide production in platelets, which might result in vasoconstriction and platelet aggregation[100,101]. Similar endothelial dysfunction, along with impairment in nitric oxide biosynthetic pathway and massive platelet activation, has been observed in COVID-19 patients[102]. Interestingly, levels of citrulline that is produced from arginine along with nitric oxide were negatively correlated with IL-6 levels in compromised COVID-19 patients, suggesting the mechanism by which cytokine storm potentiates endothelial dysfunction, especially in folate/$B_6$/$B_{12}$ deficiency[102]. Recent studies have identified homocysteine as a potential predictive biomarker in COVID-19 patients with multiple comorbidities, in addition to already established biochemical and hematological biomarkers of progression, severity and mortality in COVID-19; however, further testing is needed to determine if this finding relates to all COVID-19 patients[103,104]. Although it seems likely that COVID-19 patients might benefit from folate/$B_6$/$B_{12}$ supplementation, randomised controlled trials are certainly needed to answer.

## Microelements against coronavirus disease 2019

*Selenium.* Se is an essential trace element, well known for its requirements for optimal immune and thyroid functions. The RDA for Se is 55 μg (0·7 μmol), while the tolerable UL for adults is set at 400 μg (5·1 μmol). Se is contained in amino acid selenocysteine, positioned at the active sites of selenoproteins. Selenoprotein P is a major form of Se in plasma and, together with glutathione peroxidases, comprise nearly 90 % of the circulating Se pool, of around 85 μg/l[69,105].

Selenoprotein P, containing ten selenocysteine residues, exerts several functions, such as Se transport and storage, potent extracellular antioxidant effects, protection of endothelium by peroxynitrite scavenging and regeneration of ascorbic acid. Selenoprotein K is a cofactor in the process of post-translational protein modifications[69,105,106]. Interestingly, selenoprotein K knockout mice showed up to a 50 % reduction in most immune cell functions[105].

A rapid fall in serum selenoproteins characterises acute phase response due to a block in hepatic mRNA translation. This reaction may disrupt a Se supply to the peripheral tissues[107]. However, it does not reflect a global Se deficiency but a redistribution related to inflammation.

*The roles of selenium in the regulation of immune system functions.* Many reactions in the immune system depend on Se-containing enzymes. Se thus may influence leukocyte interactions, TLR signalling and microbicidal processes, expression and availability of inflammatory mediators and endothelial and platelet functions. Se was shown to switch pro-inflammatory macrophage phenotype (M1) towards anti-inflammatory (M2), stimulate T cell proliferation and differentiation of CD4 + Th cells, induce a stronger response to some vaccines (polio) and indirectly enhance NK cell and cytotoxic T-cell activities[105,108–112]. It is an established inhibitor of NF-kB, and thus an important transcription modulator for a variety of pro-inflammatory cytokines and chemokines[108,113].

Generally, a poor Se status reduced adaptive immunity and exacerbated inflammation. Lower plasma Se concentrations were found in the elderly, in ICU patients, with sepsis, organ failure or polytrauma, and were associated with increased mortality. In severe sepsis, the minimal plasma Se levels inversely correlated to the maximum leucocyte count, CRP and IL-6,

while directly to the minimum platelet count and antithrombin activity[113–115].

Importantly, Se deficiency was found to be a risk factor for viral infections, higher virulence, mutations and higher susceptibility to RNA viruses. Besides, a low Se status is implicated in HIV disease progression[105,113,114].

*The effects of selenium supplementation in critical illness and coronavirus disease 2019.* Se supplementation is proposed to restore immune balance after a Se deficit. For example, a prolonged high-dose Se intake (297 µg for 99 d) provoked significant changes in the leukocyte counts and responses, compared with the low Se intake (13 µg). High Se-mediated immune-enhancing properties encompassed a transient increase in lymphocyte count by 17 %, stimulated lymphocyte proliferation to a mitogen *in vitro*, improved B-lymphocyte activation/proliferation, decreased granulocyte count by 5 %, while serum immunoglobulins concentrations were largely unaffected[108]. The beneficial effects are thought to reside in the restoration of cellular redox control. Both organic and inorganic Se supplementation (50 µg for 28 d) induced an increase in phospholipid and cytosolic glutathione peroxidases activities in lymphocytes, granulocytes and platelets[116]. Se supplementation also restored the antioxidant capacity of the lungs and improved respiratory mechanics[117].

However, there is no clear suggestion over Se supplementation during critical illnesses[109]. In a meta-analysis of Heyland *et al.*[118], Se supplementation, alone or in combination with other antioxidants, was associated with reduced mortality, while other antioxidants were not. There is an increasing number of studies reporting a trend towards better outcomes in Se supplemented COVID-19 patients[113–115,119].

Also, several of them determined a higher risk for severe COVID-19 and related mortality in SE-deficient patients[113–115,119]. Low Se serum levels were associated with the severity of COVID-19 in a South Korean study. Se deficiency (< 95 ng/ml) was present in 44·4 % of cases with a mild disease without pneumonia, opposed to 100 % of those with severe pneumonia requiring mechanical ventilation[12]. A study from July 2020 showed an insufficient Se status (< 2·5th percentile of a reference European population or < 45·7 µg/l) and Se availability for the optimal selenoproteins expression in COVID-19 patients. Additionally, Se status was significantly higher in surviving COVID-19 patients when compared to non-survivors[114].

Population-based, retrospective analysis of Zhang *et al.* reported an association between Se status and cure/death rate from COVID-19 in the Chinese population. Despite the limitations, this study points towards the need for further research, particularly in light of associations between Se status and other viral disease outcomes[115].

There may be a specific role for Se in disease caused by RNA viruses. Through the increase in oxidative stress, Se deficiency possibly increases the mutation rate of the viral genome and virulence of the viral agent[120]. RNA viruses synthesise their selenoproteins and thus are likely to exploit cellular Se for replication and survival, causing Se deficiency in the host cell. In this situation, Se supplementation would compensate for the lost quantities and restore redox balance and immune defense[113,120].

It is also important to note that SE deficit may predispose to endothelial dysfunction and platelet activation, both leading to coagulopathy, an established complication of COVID-19. Preclinical studies have demonstrated that sodium selenite has a reversal effect on platelet aggregation, acting through the reduction of lipid peroxidation and thromboxane A2 formation[121,122].

To counteract the SARS-CoV-2 infection, there is a suggestion for Se supplementation in doses well above RDA (200–400 µg/d), based on the previous studies on severe viral infections and good tolerance of the high Se doses given in a short period (2–3 weeks)[113].

## Zinc, copper and iron

*Nutritional immunity.* Trace elements such as Zn and Cu are required for the optimal function of the immune system, but are considered essential for the growth and survival of various pathogens as well. The competition between the host and the pathogen leads to defensive sequestration of trace minerals during infection and uses the toxicity of these metals against the pathogen in a process called nutritional immunity[123]. Metallothioneins are a group of cysteine-rich low molecular weight proteins, with a metal-binding ability that sequestrates Zn and Cu in unstressed cells and regulates Zn/Cu signalling during immune cells activation[124,125]. However, they might also sequestrate heavy metals, scavenge reactive oxygen and nitrogen species and regulate cellular redox potential[124,126]. Extracellular metallothioneins act as danger signals because they could induce chemotaxis and influence the immune response[124].

*Zinc.* Zn is an essential micro-nutrient that catalyses enzyme activity, contributes to protein structure and regulates gene expression. RDA for Zn is 11 mg for males and 8 mg for females, respectively. Deficiency is not common; however, vegetarians, pregnant and lactating women, alcoholics and individuals with chronic diseases might be at risk of inadequacy[52,127].

Zn homoeostasis is controlled through the action of Zn transporters and Zn-binding proteins and is essential for maintaining the membrane barrier structure, cell differentiation, cell division and the proper function of immune cells[128,129].

*Implications for the use of zinc in coronavirus disease 2019.* Zn contributes to the host defense by maintaining the membrane barrier structure and function[129].

During infection or inflammation, plasma Zn levels are reduced due to the effect of IL-6 that upregulates the expression of Zn transporters in hepatocytes and the accumulation of metallothionein-bound Zn in the liver[130]. Prophylactic Zn supplementation demonstrated a survival advantage and reduced neutrophil infiltration in the lungs of mice in a murine model of sepsis and acute lung injury[131,132]. Additionally, low Zn levels result in increased release of NET and enhanced neutrophils degranulation, whereas Zn supplementation inhibits NET release and degranulation in both human and murine neutrophils by inhibiting citrullination of histone H3[132,133]. Although

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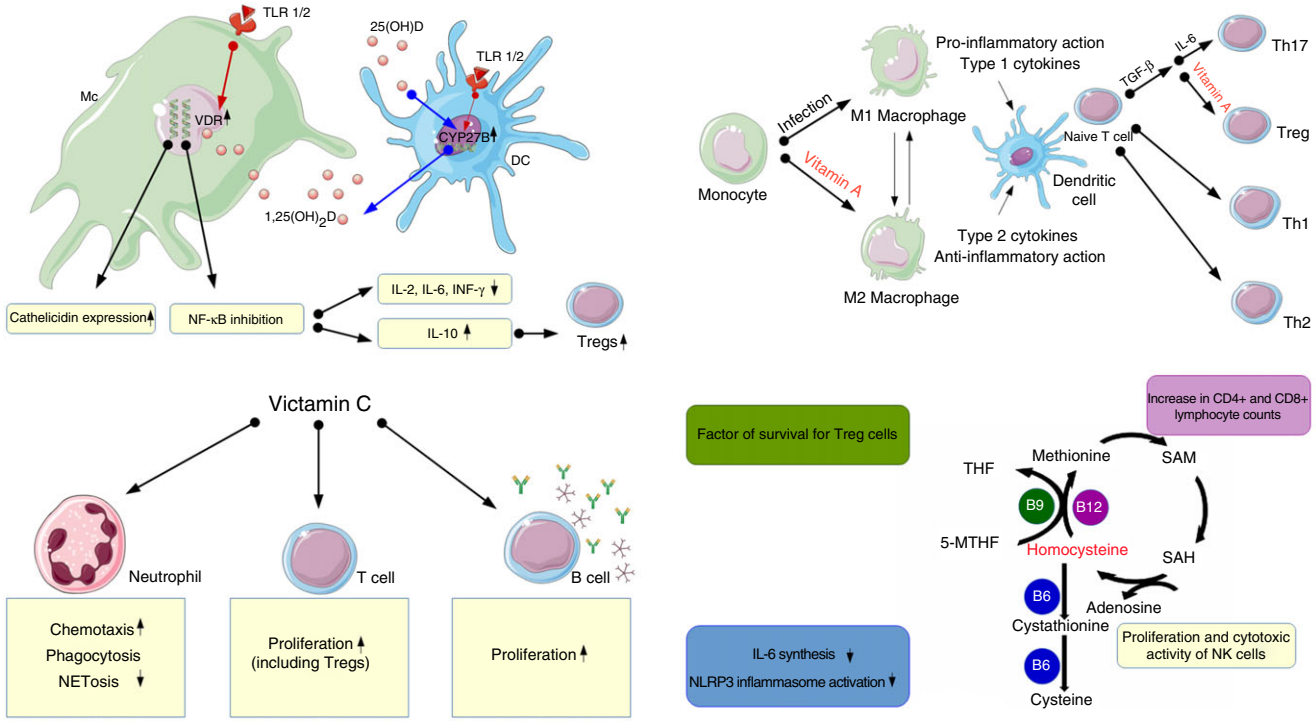


**Fig. 1.** Mechanisms of vitamins beneficial effects in the pathogenesis of COVID-19. Mc, macrophages; Ly, lymphocytes; Tregs, T regulatory lymphocytes; TLR ½, toll-like receptor 1/2; VDR, vitamin D receptor; 25OHD, calcidiol; 25(OH)2D, calcitriol; CYP27B1, Cytochrome P450 Family 27 Subfamily B Member 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin; INF-γ, interferon γ; M1, pro-inflammatory macrophage phenotype; M2, anti-inflammatory macrophage phenotype; TGF-β, transforming growth factor-beta; NET, neutrophil extracellular traps; DC, dendritic cell; Ab-antibodies; SAM-S-adenosyl methionine; SAH-S, adenosyl-L-homocysteine; 5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; NK cells, natural killer cells. Red/blue arrows – stimulation. This figure was drawn using the vector image bank of Servier Medical Art (http://smart.servier.com/). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3·0 Unported License (·https://creativecommons.org/licenses/by/3·0/).

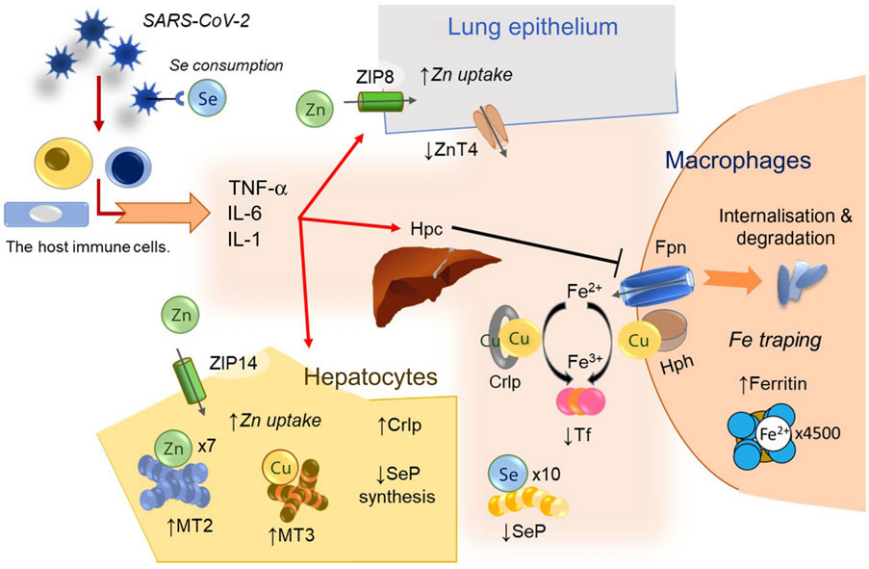


**Fig. 2.** Mechanisms of microelements sequestration (iron, copper, zinc and selenium). Red arrows, stimulation; block arrow, inhibition; Hpc, hepcidin; Hph, hephestin; Fpn, ferroportin, Tf, transferrin; Crlp, Ceruloplasmin; SeP, selenoprotein P; ZIP, Zrt/Irt-like protein (mediate Zn influx); ZnT, zinc transporters (mediate Zn efflux); MT, metallothioneins.

the physiologic concentration of Zn did not affect cytokine expression or apoptosis of peripheral blood mononuclear cells, pharmacologic concentrations stimulated both cytokine expression and apoptosis *in vitro*[134].

It was shown previously that an increase in the intracellular Zn concentration impairs the replication of a variety of RNA viruses, including poliovirus, influenza and SARS-CoV virus[135]. Therefore, due to the potential effect on SARS-CoV-2 replication

and the effect on the NET formation, Zn supplementation might exert highly protective effects in COVID-19 patients and especially in the pathogenesis of multiorgan failure. However, supraphysiological doses of Zn might bust cytokine production and aggravate cytokine storm.

*Zinc in the therapy of coronavirus disease 2019.* Zn supplementation was previously associated with reductions in the rates of pneumonia in children aged up to 5 years in low-income areas[136]. The addition of Zn to the standard treatment and care was proven beneficial in infants with a severe bacterial infection in a randomised controlled trial[137]. Additionally, Zn deficiency was associated with more complications, prolonged hospital stay and increased mortality in COVID-19 patients[138]. Observational and cohort studies regarding the use of Zn in COVID-19 patients showed promising results, reporting reduced mortality among hospitalised patients, especially when administered together with Zn ionophore[139,140]. However, the study involving ambulatory COVID-19 patients recently showed that 50 mg of Zn gluconate for 10 days did not affect the severity or duration of symptoms compared with usual care[77]. Additionally, supplementation with 80 mg of Zn did not reduce the risk of infection with the SARS-CoV-2 virus[141].

Still, patients with COVID-19 that require mechanical ventilation might benefit from Zn supplementation, and there is a need for further studies. Low serum values of Zn are found in critically ill patients with COVID-19, especially in those who require invasive mechanical ventilation and correlate with clinical presentation and worse outcomes[138,142,143]. Mechanical ventilation is often necessary to support patients with the severe presentation of COVID-19. However, it also might exacerbate lung injury through mechanical stress-activated signalling pathways[144]. It was shown that stretch applied to cultured human cells and mouse lungs *in vivo* induces expression of metallothionein, which requires Zn to limit lung injury[145]. Additionally, Zn deficiency potentiated the development of ventilator-induced lung injury in mice[145]. On this subject, further clinical research is needed since ventilator-induced lung injury is commonly present among ICU patients with acute respiratory distress syndrome. Currently, there are published protocols for registered RCT that are to test the effects of intravenous Zn administration in critically ill patients[146].

*Copper.* Cu is required for the proper function of both innate and adaptive immune responses.

Physiologically, in acute phase response, Cu plasma concentrations increase as a result of increased ceruloplasmin synthesis and are thought to provide an antioxidant defense[147]. Cu deficiency is reflected in reduced neutrophil count, as well as in impaired neutrophil and macrophage function reduced cytokine synthesis and T cell proliferation, but is rare in humans[148,149]. Cu, being transitional metal, increases reactive oxygen and nitrogen species generation during the respiratory burst, thus potentiating the antimicrobial effect excreted by neutrophils and macrophages[150]. A recent preprint suggested that Cu might inhibit coronavirus main protease (Mpro) by docking cysteine (Cys145) in the Mpro active-site region[151].

*Iron.* In addition to Cu, Fe, as well causes nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst in macrophages and neutrophils, which can be inhibited by Fe chelation[152]. Both increase and decrease in the intracellular Fe level act as danger signals and activate inflammatory and anti-microbial pathways through NF-κB and HIF-1, respectively[153]. Cytokines such as IL-6 and the acute phase protein hepcidin lead to the retention of Fe within macrophages in the form of ferritin and a sequential decrease in serum Fe concentration[154].

Fe metabolism might play an important role in COVID-19. Severe COVID-19 cases had lower haemoglobin, red blood cell count and higher ferritin levels when compared with moderate cases, whereas ferritin levels were higher in non-survivors when compared with survivors[155]. Fe overload has been proposed as a contributor to the pathogenesis of COVID-19 due to its role in the generation of reactive oxygen species, but possibly more important due to induction of nonapoptotic cell death ferroptosis[79,156]. The process of ferroptosis is characterised by the Fe-dependent accumulation of lethal lipid reactive oxygen species that promotes inflammation, but can be inhibited by Fe chelators and lipophilic antioxidants such as tocopherols and carotenoids[79,157].

*Interactions of zinc, selenium, copper and iron: implications for the supplementation in coronavirus disease 2019 patients.* Regarding supplementation, interactions of Zn with other trace metals and vitamins should be considered. Absorption and bioavailability of Zn, Cu and Fe are dependent on competition among elements and, when given together in equal ratio, absorption of Fe and Cu is reduced approximately 40 %[158]. Therefore, excessive Zn supplementation might result in Cu or Fe inadequacy. Both Cu and Zn bind to metallothioneins, but Cu binds with higher affinity that results in the formation of mixed metal–metallothionein complexes and the release of Zn[159]. However, Se compounds catalytically couple with the glutathione/glutathione disulphide and metallothionein/thionein redox pairs to either release or bind Zn, thereby expressing antioxidant or prooxidant effects through redox catalysis in Zn metabolism[160,161].

## Conclusion

One year after the COVID-19 pandemic was proclaimed by WHO, we are still learning about the disease. Therapeutic protocols have been evolving and improving ever since. Finally, since the beginning of 2021, vaccines have become available, but the roll-out has not been as fast as expected in some countries. Meanwhile, vitamins and microelements have been widely used as supplements in prophylaxis and the fight against COVID-19. Even though several studies reported a promising association of these micronutrients on the reduction of severity and improved survival, up to date there is not enough scientific evidence neither for nor against their use in the treatment of COVID-19. Some of them, i.e., vitamin D, vitamin C and Zn, have been widely prescribed by medical professionals, sometimes in supraphysiological or bolus doses, disregarding potential side effects and interactions. Although the use of some micronutrients in the COVID-19 therapy might be scientifically sound, the

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potential beneficial effects should be evaluated in RCT first. From the current standpoint, vitamin and microelement supplementation in COVID-19 should not exceed the doses recommended for the general population and age group, except for clinical trials. Inevitably, vitamin D, Zn and Se show a significant place in the management of COVID-19, especially in populations prone to deficiencies. Besides, regular intake of recommended doses of vitamin D should be encouraged in healthy people to maintain a well-balanced immune system. Given the importance of providing optimal resistance to SARS-CoV-2, maintaining a balanced diet, in line with necessary vitamin and microelement substitutions, represents a feasible, safe and readily available therapeutic option.

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