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ASSOCIATION OF VITAMIN D WITH CORONAVIRUS DISEASE 2019 (COVID-19): A REVIEW

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Abstract

Nutrition is essential for the function of the immune system and this relationship is currently being studied. In particular, vitamin D is a crucial immunonutrient that can also be obtained through the diet. Although the primary function of vitamin D appears to be calcium homeostasis, this vitamin also serves for immunomodulatory functions. The link between vitamin D deficiency and susceptibility to infections originated more than a century ago when vitamin D was used for the treatment of tuberculosis. The importance of vitamin D in cases of respiratory infection is supported by the fact that low levels of vitamin D are common in populations and low levels have been associated with a significantly increased risk of pneumonia. Vitamin D supplementation can improve innate immunity as well as adaptive immunity. The rapid global spread of COVID-19 has renewed interest in the possible role of vitamin D in modulating the immune response to respiratory infections.

Keywords: *Vitamin D, Covid-19, Association, Review, Respiratory infection.*

Introduction

The COVID-19 pandemic is a health threat with unprecedented consequences worldwide. To date, various factors have been identified as predisposition for an aggressive Covid-19 phenotype, including male gender, age>65, smoking and comorbidities such as diabetes, hypertension and cardiovascular disease [1].

Most of these comorbidities are associated with a sedentary lifestyle and an unhealthy diet [2]. Nutrition is also essential for the function of the immune system and this relationship is currently being studied. In particular, vitamin D is a crucial immunonutrient that can also be obtained through the diet. Although the primary function of vitamin D appears to be calcium homeostasis, this vitamin also serves for immunomodulatory functions.

The rapid global spread of COVID-19 has renewed interest in the possible role of vitamin D in modulating the immune response to respiratory infections. Indeed, widespread vitamin D supplementation has been proposed as a preventative health measure [3].

The relationship between Vitamin D and Covid-19 is still in many controversies and uncertainties, both for the scientific community and for doctors, despite the fact that the number of documents on this subject is constantly growing. This review is mainly based on a literature search as it is necessary to identify and summarize the key aspects known to date as well as to provide the main implications for clinical practice.

COVID-19

Coronavirus Disease 2019 (COVID-19) was declared as pandemic by the World Health Organization on March 11th, 2020 mainly due to the speed and scale of the transmission of the disease [4].

Before that, it started as an epidemic in mainland China with the focus being firstly reported in the city of Wuhan, Hubei province in February 26th [5, 6, 7].

The pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the Betacoronavirus family, which also includes MERS-CoV and SARS-CoV [8, 9]. The latter shares ~75–80% of its viral genome with SARS-CoV-2 [10,11].

Beta-coronaviruses have three important envelope proteins: Spike (S) protein, Membrane (M) protein, and Envelope (E) protein. S protein mediates viral attachment to the cell membrane receptor, membrane fusion, and ultimately viral entry into the host cell. M protein, the most abundant membrane protein, together with E protein are responsible for the coronavirus membrane structure. Another component of the beta-coronavirus is the N protein, which is the protein component of the helical nucleocapsid that includes the genome RNA [12, 13]. The Severe Acute Respiratory syndrome virus (SARS-CoV-2) induces an inflammatory state, evidenced by raised acute markers like interleukin-6 (IL-6), c-reactive protein (CRP), ferritin, d-dimer etc. and may lead to lung damage especially in the second week of illness owing to the 'cytokine storm' [14,15].

Patients suffering from SARS-CoV-2 infection usually presented with fever, dry cough, upper airway congestion, sputum production, and shortness of breath, but rarely headache, hemoptysis, and diarrhea. Loss of smell (*anosmia*) and loss of taste (*ageusia*) have also been reported [16,17].

Vitamin D

Vitamin D is a fat-soluble vitamin that is produced for about 80-90% in the skin in the presence of solar ultraviolet rays (UV) and for the remaining 10-20% is taken with the diet.

Regardless of the mode of intake, it requires further modifications to become the biologically active form. Vitamin D has always been known as the vitamin that regulates bone metabolism. In fact, already in the nineteenth century the deficiency of this vitamin was identified as the cause of the rickets epidemic that affected children living in industrial cities. Vitamin D is present in two forms: vitamin D2 (ergocalciferol) resulting from the irradiation of sterol ergosterol, contained in yeasts and plants, and vitamin D3 (cholecalciferol) present in some foods and synthesized by the skin when

exposed to the sun. Foods that contain vitamin D are few and are egg yolk, cod liver oil and fatty fish such as salmon, mackerel and herring.
The skin synthesis of vitamin D occurs instead by conversion of 7 dehydrocholesterol into pre-vitamin D3 and then into vitamin D3 (cholecalciferol) under the effect of ultraviolet rays B. However, excessive exposure to the sun degrades previtamin D3 and vitamin D3 in inactive photoproducts.
In humans, typically only 10% of the necessary vitamin D is taken through food, while endogenous synthesis is prevalent (90%). For this reason, vitamin D is also called "sunshine vitamin".
Vitamin D absorbed from the intestine or that synthesized in the skin, is present in the circulation linked to the vitamin D binding protein, an α globulin synthesized in the liver. In the liver, vitamin D is subsequently hydroxylated at position 25 by mitochondrial and microsomal enzymes similar to cytochrome P450. The activity of this enzyme is not subject to strict regulation and the resulting metabolite 25-hydroxycholecalciferol or calciferol or 25-hydroxyvitamin D (25OHD) is the main circulating and storage form of vitamin D. Approximately 88% of 25 (OH) D circulates bound to the specific binding protein, 0.03% is in free form and the remaining portion is bound to albumin. The half-life of 25 (OH) D is approximately 2-3 weeks, however it is significantly shorter when the levels of the specific binding protein are reduced, as in the case of increased urinary loss in nephrotic syndrome.
25 (OH) D must undergo subsequent hydroxylation to transform into the active form of vitamin D called calcitriol or 1,25 (OH) 2D dihydroxyvitamin D. This final hydroxylation occurs in the kidney by the 25 (OH) -1α-hydroxylase expressed in the cells of the proximal convoluted tubule and subjected to strict regulation. Parathyroid hormone (PTH) and hypophosphatemia stimulate this enzyme, while calcium and the product of the enzymatic reaction 1.25 (OH) 2D inhibit it. The main route of inactivation of the metabolites of vitamin D is an additional hydroxylation by the enzyme 24-hydroxylase, expressed in most tissues. 1.25 (OH) 2D is the main inducer of 24-hydroxylase and thus promotes its inactivation, limiting its biological effects. The metabolites are secreted into the bile and reabsorbed by the enterohepatic circulation (alterations of this circulation such as terminal ileum lead to loss of vitamin D metabolites) [18].

Vitamin D and respiratory infection

The link between vitamin D deficiency and susceptibility to infections originated more than a century ago when it was observed that children with rickets were more prone to respiratory infections [19]. Vitamin D was subsequently used, before the discovery of antibiotics, for the treatment of tuberculosis [20]. More recently epidemiological studies have shown a strong association between seasonal variations in vitamin D and the incidence of infectious diseases such as sepsis [21], respiratory infections [22] and flu [23].
Vitamin D appears to act against bacterial infections by stimulating the production of antimicrobial peptides such as cathelicidin and beta difensin [24, 25] and against viral infections by suppressing the excessive production of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin 12 (IL-12) [25]. In particular, when the cells of the monocyte-macrophage line come into contact with an infectious agent such as mycobacterium tubercolosis, they increase the expression of the gene for VDR and the gene for 1-α- hydroxylase. This involves the increased local production of 1,25 (OH) 2D which stimulates synthesis by autocrine way of cathelicidin which is able to destroy the infectious agent [23]. If there is a 25 (OH) D deficiency, macrophages do not initiate the innate immune response. This may explain why black Americans, who are often more vitamin D deficient, are more susceptible to tuberculous infection than whites [26]. In a large US series, an inverse correlation was observed between the incidence of upper respiratory tract infections and serum levels of 25 (OH) D [27]. The association was particularly evident in patients with chronic obstructive pulmonary disease or asthma, in which, among other

things, a positive correlation has recently been observed between serum levels of vitamin D, respiratory function and response to corticosteroids [28].
Recently, in a randomized, double-blind placebo trial, it was observed that the administration of 1200 IU of vitamin D3 / day in school-aged children can reduce the risk of getting influenza A by more than 40% [29].

Consumption monitoring in Italy

With Note 96, published on 26 October 2019, the Italian Medicines Agency (AIFA) redefined the conditions for the prescription by the National Health Service of vitamin D-based drugs classified in band A (reimbursable by the NHS) - cholecalciferol, cholecalciferol / calcium salts and calcifediol - exclusively for the prevention and treatment of Vitamin D deficiency in the adult population (> 18 years)(Figure 1).
In the first three months of application of Note 96 (November 2019 / January 2020). There was an overall decrease in consumption and expenditure of drugs in the Note of over 30% compared to previous periods both in terms of packs supplied and expenditure incurred by the NHS.
There are no significant increases in the consumption and expenditure of other Vitamin D analogues not covered by the Note. The 40-50 age group is the one that recorded the greatest reduction in consumption, especially among women.
The impact of the Note at the level of the different regions is to be considered heterogeneous both for the different regional scenarios before the application of the Note, and for the different responses observed on the territory after the application of the provision.
To reduce regional heterogeneity and to maintain the persistence of the effect of the intervention over time, it is necessary to promote initiatives also in the area aimed at raising awareness among health professionals and prescribers on the appropriate use of Vitamin D and similar [30].
In the first fifteen months of application of note 96 (November 2019 - January 2021) there was an overall decrease in consumption and expenditure of drugs in the note of almost 30% (over 117 million in absolute terms) compared to the previous periods both in terms of packs supplied and expenditure incurred by the National Health Service, with an average monthly saving of approximately 7.8 million euros.
This is what emerges from the monitoring carried out by the Agency to verify the effects of the application of Note 96, through the analysis of national and regional data.
The most important effect in economic terms of the Note occurred in the first 12 months with an average saving of 9.1 million / month; currently, the residual effect (in months 13-15) can be estimated at a reduction of about
2.1 million / month.
The stable trend in consumption and spending in the last quarter confirms the persistence of the long-term effectiveness of the Note. On the other hand, there are no significant increases in the consumption and expenditure of other Vitamin D analogues not covered by the note. Finally, the heterogeneity of the impact of the note at the level of the different regions is confirmed [31].

VITAMIN D AND COVID-19

In COVID-19 patients, disease severity is often determined by the onset of complications such as pneumonia
/ acute respiratory distress syndrome (ARDS), myocarditis, microvascular thrombosis and / or cytokine storm, all of which involve underlying inflammation. While Covid-19 specific CD8 T cells and specific antibodies produced by B cells are key to eliminating the virus. Non-specific and uncontrolled inflammation with subsequent release of cytokines has been shown to cause catastrophic damage to the lungs and other vital organs. Consequently, the reduction of this early non-specific inflammation during the early symptoms of Covid-19 may provide time for the development of specific acquired immunity against severe disease [32].
The course of SARS-CoV-2 infection from infection to symptoms is about five days, so after symptoms develop, there are about seven days when these symptoms become increasingly

progressive. About 20% of symptomatic patients require hospitalization, while most will actually be better off, largely thanks to a robust response of the innate immune system during the early stage of the disease [33].

Overall, when pathogens enter the body, the first to respond is innate immunity followed by adaptive immunity [34].

Vitamin D supplementation can improve innate immunity [35, 36, 37] as well as adaptive immunity [38, 39]. Since antigen-presenting cells (macrophages and dendritic cells) can synthesize 1,25 (OH) 2D from 25 (OH) D, it has been hypothesized that vitamin D supplementation might improve the function of antigen-presenting cells, thus improving the overall immune response [40](Figure 2).

A primary defense against uncontrolled inflammation, and viral infection in general, is provided by regulatory T cells (Tregs) [41].

In a study of elderly patients in nursing homes, high blood levels of Treg were found to be associated with a reduced level of respiratory viral disease [42].

These observations suggest that if Treg levels can be increased, this could be of benefit in decreasing the severity of viral disease and possibly COVID-19. Treg levels can be increased with vitamin D supplementation [43, 44].

The importance of vitamin D in cases of respiratory infection is supported by the fact that low levels of vitamin D are common in populations around the world and low levels have been associated with a significantly increased risk of pneumonia [45] and viral infections of the upper respiratory tract [46].

Vitamin D deficiency (serum 25-hydroxyvitamin D (25 (OH) D) <50 nmol / L) is present in 30-60% of the populations of Western, Southern and Eastern Europe and up to 80% of the populations of Middle Eastern countries [47]. In addition, an even more severe deficiency (serum levels <30 nmol / L) is reported in over 10% of Europeans. Low vitamin D levels are also associated with an increase in inflammatory cytokines. A study of healthy women in the United States found a significant inverse relationship between serum levels of 25 (OH) D and TNF-alpha [48]. In another report, IL-6 levels were found to be increased in those who were vitamin D deficient.

In a wide variety of animal studies and in vitro cell models, vitamin D3 has been shown to reduce the production of inflammatory cytokines, such as TNF-alpha and IL6, while increasing inhibitory cytokines [49]. These studies raise the possibility that adequate levels of vitamin D can reduce the incidence of the cytokine storm, which can occur in COVID-19. Thrombotic complications are common in patients with COVID-19 [50]. Of those with severe disease, more than half experienced elevated D-dimer levels. Interestingly, vitamin D is also involved in the regulation of thrombotic pathways and vitamin D deficiency is associated with an increase in thrombotic episodes [51, 52](Figure 3).

Vitamin D deficiency has also been found to occur more frequently in patients with obesity and diabetes. These conditions are reported to lead to higher mortality in COVID-19. An increased risk of death with COVID-19 is also seen in black, Asian and ethnic minority (BAME) groups. Since melanin reduces vitamin D production associated with exposure to ultraviolet radiation from sunlight, this may help explain the frequent observed occurrence of vitamin D deficiency in BAME groups [32].

A further interesting point is represented by the potential differences in serum level of 25(OH)D among men and women. Sanghera and co-workers [53] observed a significantly reduced level of 25(OH)D in both men and women with obesity that represents a further important risk factor for COVID-19. In this study, 25(OH)D level remains consistently lower in obese men than in obese women [50]. On the contrary, in another study, Mucogiuri and co-workers [54]. Stratifying the sample population according to sex and body mass index (BMI), found that 25(OH)D concentrations were significantly higher in males compared to females in all BMI classes and decreased along with the increase of BMI values. Although these contrasting data seem to not assign to 25(OH)D a clear role in determining sex differences in obese COVID-19 patients, we think that attention could be paid to 25(OH)D levels in the context of this comorbidity.

Interestingly, Spanier and co-workers [55] suggested that Vitamin D3 acts in an estrogen-dependent manner in controlling T regulatory cell differentiation. Moreover, estrogen seems to increase the expression of the nuclear vitamin D receptor (VDR) gene in CD4+ T cells [56] and to decrease the expression of CYP24A1, the cytochrome P450 component of the 25-hydroxyvitaminD(3)-24-hydroxylase enzyme which inactivates Vitamin D3. In turn, Vitamin D3 exerts tissue-specific effects on peripheral estrogen metabolism [57]. Hence, the sex-related immunomodulatory effects of Vitamin D3 suggest that it is possible to speculate that also in COVID-19, Vitamin D3 could play a role in the outcome and lethality [58].

Furthermore, a recent study has shown that vitamin D levels in critically ill COVID-19 patients and found a correlation with inflammatory markers: patients who had low vitamin D levels also had serum levels of IL-6, TNF-α and ferritin significantly higher. [59] Vitamin D levels were significantly lower in severe COVID-19 patients and the mortality rate was very high in patients with vitamin D deficiency. However, a recent clinical study has shown that supplementation with a single very high dose of 200,000 IU of which increased serum levels of vitamin D (21-44 ng / mL) was however ineffective in reducing the length of hospital stay or any other clinical outcome among hospitalized patients with severe COVID-19 [60].

Therefore, although not necessarily causative, these results show that low vitamin D levels are often associated with worse severity of COVID-19 infection [60-65], with moderate to high vitamin D supplementation effective in reduce the severity of COVID-19. and mortality.

So based on these findings, it is likely to be helpful to take vitamin D supplements, especially in the winter months, to better control seasonal flu, the common cold, the flu, and ongoing COVID 19. It may be advisable for doctors to measure circulation levels of vitamin D and, if the level is below normal, recommend vitamin D supplementation and / or sufficient exposure to sunlight. Based on available guidelines, the threshold for healthy serum 25 (OH) D is ~ 30 ng / mL to maintain optimal serum calcium levels [133-136]. Below 20 ng / mL (50 nmol / L) it is considered vitamin D deficient, while 21 - 29 ng / mL (52.5 - 72.5 nmol / L) is vitamin D deficient [66].

For an immunomodulatory effect, a serum level of 30 ng / mL of 25 (OH) D has been suggested to be essential [64,65]. However, what is the optimal serum level of 25 (OH) D to maximize its effect on the immune system against SARS-CoV-2 infection remains questionable.

A cross-sectional study with 235 COVID-19 patients showed that serum 25 (OH) D levels of at least 30 ng / mL were associated with a significant reduction in clinical outcomes and COVID-19 mortality [20]. Therefore, overall, a desirable serum level of 25 (OH) D of at least 30 / mL appears to be more useful for COVID-19 patients [66,69]. It is important to recognize that there is potential for vitamin D toxicity. As a result, more than the usual daily supplement should only be taken under medical supervision. The efficacy of vitamin D supplements in preventing acute respiratory tract infections has been best demonstrated with chronic low-dose intake rather than large-dose bolus administration [70].

Instead, a single large dose failed to improve the outcomes of patients with vitamin D deficiency admitted to the intensive care unit (ICU) with pneumonia, sepsis, shock, or respiratory failure, compared to ICU patients who were given it. a placebo [71]

Recurrent vomiting, abdominal pain, polydipsia, polyuria, confusion and apathy are the most frequently observed clinical symptoms of acute vitamin D toxicity (VDT) or vitamin D intoxication or hypervitaminosis D [72]. The Endocrine Society and the Institute of Medicine (IOM) have both stated that acute VDT is extremely rare [73,74]. Although rare, if not identified quickly, the health effects can be severe. Serum concentrations of 25 (OH) D above 150 ng / mL (375 nmol / L) are the hallmark of VDT and levels have been found to cause hypercalcemia [75]. Long-term daily consumption of vitamin D greater than 40,000 IU (1000 µg) has been found to cause hypercalcemia in healthy people [76]. Hypercalcemia has a direct relationship

with serum 25 (OH) D levels but not with 1,25 (OH) 2D levels [52,77].

CONCLUSIONS

Vitamin D is almost undoubtedly associated with several viral infections, including COVID-19, although the mechanism of this link is still a field of study. The multitude of data collected to date assumes that higher doses of vitamin D may be potentially beneficial for the majority of COVID-19 patients. For this reason, adequate vitamin D supplementation must be implemented in populations where vitamin D deficiency is prevalent, contributing to the prevention of respiratory infections and its complications.
Further studies could support the thesis of integrating vitamin D in any form of therapy thanks to its potential anti-inflammatory and immunomodulatory effects.

Conflicts of interest. — The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript

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Figure 1. AIFA Note 96

DRUGS INCLUDED IN THE AIFA NOTE: ***CHOLECALCIFEROL*** ***CHOLECALCIFEROL / CALCIUM SALTS*** ***CALCIFEDIOL***	**The prescription by the NHS of drugs with the indication "prevention and treatment of vitamin D deficiency" in adults (> 18 years) is limited to the following conditions:** ➢ **Prevention and treatment of vitamin D deficiency in the following clinical scenarios:** **<u>regardless of the determination of 25 (OH) D</u>:** - **institutionalized people** - **pregnant or lactating women** - **people with osteoporosis of any cause or known osteopathies not candidates for remineralizing therapy (see note 79)** **<u>after determining the 25 (OH) D</u>:** - **people with serum levels of 25OHD <20 ng / mL and symptoms attributable to hypovitaminosis (asthenia, myalgia, widespread or localized pain, frequent unjustified falls)** - **people diagnosed with hyperparathyroidism secondary to hypovitaminosis D** - **people with osteoporosis of any cause or known osteopathies candidates for remineralizing therapy for which the correction of hypovitaminosis should be a prerequisite for the start of therapy *** - **a long-term therapy with drugs that interfere with the metabolism of vitamin D** - **diseases that can cause malabsorption in adults** *** Remineralizing therapies should be started after correction of hypovitaminosis D**

Figure 2. Schematic representation of a possible correlation between Vitamin D deficiency and COVID-19 severity

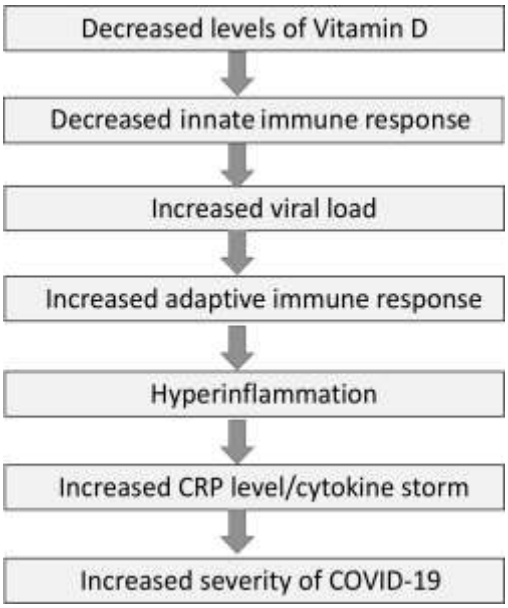

Figure 3. Biochemical correlations between COVID-19 and vitamin D deficiency

PARAMETERS	COVID-19	VITAMIN D DEFICIENCY
IL-6	Increased	Increased
TNF-α	Increased	Increased
INF-γ	Increased (late in course)	Increased
C-reactive protein	Increased	Increased
D-dimer	Increased	Increased
Innate immune response	Decreased	Decreased
Th1 adaptive immune response	Increased (late in course)	Increased
Cytokine storm	Increased	Increased
ACE2 expression	Decreased	Decreased
Coagulability	Incresead	Increased