VITAMIN D
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Vitamin D, risk of infection with SARS-CoV-2 and severity of COVID-19: doubts, possibilities and evidence

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INTRODUCTION

Vitamin D is a key regulator for the development and maturation of all immune system lineages. In cases of deficiency, supplementation has shown positive effects in acute respiratory infections, even though it does not reduce the incidence of serious events.

Many reports, based on observations made during the first pandemic wave in Italy, suggest an association between vitamin D deficiency, risk of infection with SARS-CoV-2, incidence and severity of COVID-19, and mortality. Speculative observations have proposed a correlation between the fact that Italy has the highest prevalence of hypovitaminosis D among European countries and that the country experienced a very high incidence of infections with SARS-CoV-2 and COVID-19, above all in the northern regions. These studies, however, associate the two events without verifying the causal nexus and without excluding random factors. Vitamin D status, infection risk and development of serious pathological forms are complex phenomena which depend on countless variables, whose multifactorial relations of interdependence cannot be described by their mere summation. For this reason, only large cohort studies, which do not ignore fundamental variables, can take on epidemiological relevance [1].

ROLE OF VITAMIN D IN INNATE AND ADAPTIVE IMMUNE RESPONSES

Vitamin D plays significant roles in connection with innate immunity by means of antimicrobial action (regulation of iron metabolism, autophagy and enhancement of the epithelial barrier function, oxidative stress, induction of antimicrobial gene expression – defensins and cathelicidins – and toll-like receptors), adaptive response modulation and tolerance induction [1]. More specifically, 1,25(OH)2D carries out antimicrobial activity on its own, in that it is able to induce the expression of cathelicidin and β-defensin 2, proteins with antimicrobial effectiveness, both direct and indirect (by stimulating the chemotaxis of cells of the immune system, by inducing the expression of proinflammatory cytokines and by effecting the removal of infected cells in the respiratory tract). Vitamin D also stimulates β-defensin 2 expression through the induction of the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) [2]. In addition, $1,25(OH)_2D$ inhibits the expression of hepcidin and therefore suppresses the hepcidin-mediated block of iron export through ferroportin: the net result is an increased outflow of iron from the infected cell and, consequently, a reduction of the availability of this element for microbial growth [3].

In fact, the antimicrobial effects of vitamin D are several: they also include stimulation of the barrier function of the intestinal [4] and alveolar [5] epithelia, of the production of reactive oxygen species (ROS) [6], of the neutrophilic function [7] and of the phagocytic and auto phagocytic activity (through the induction of the key effectors of autophagy: LC3, beclin 1 and PI3Kγ3) of macrophages [8]. Both the induction of cathelicidins and defensins and the stimulation of the pro-autophagic pathways in cells with antigen have significant antiviral effects: respectively, they inhibit the replication of viruses [9] and aid in the clearance of viral particles [10]. In connection with adaptive immunity, calcitriol limits the activation of T lymphocytes [11] and induces the expression of regulatory phenotypes (Treg)

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Conflict of interest
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which mediate immune tolerance and limit abnormal immune responses as well as the phenotypic shift from T helper Th1/Th17 to Th2 (from proinflammatory to regulatory) [12].
The effectiveness of vitamin D action is a function of the activity of its receptor, VDR. In fact, single-nucleotide polymorphisms (SNPs) in the VDR gene affect protein responsiveness and have been associated with a number of immune dysfunctions: compared to the CT and CC genotypes, the TT genotype of the FokI polymorphism, for example, has been associated with greater risk of infection with respiratory syncytial virus (RSV) [13].

VITAMIN D AND RISK OF INFECTION WITH SARS-COV-2

The hypothesis of a role of vitamin D in the risk of infection with SARS-CoV-2 stems in part from the observation of a high prevalence of hypocalcemia (50%) among patients hospitalized during the Ebola (2016) and SARS (2003) epidemics. Up to 80% of COVID-19 patients hospitalized in Italy during the first wave had [Ca^{2+}] < 1,18 mmol/L. Free calcium is necessary for virus-cell interaction (through the spike protein and ACE2), viral replication and the inflammatory response to the infection. The correlation between vitamin D status and risk of infection may be – at least in part – a result of the deregulation of calcium and phosphate homeostasis [14]. That calcium plays a fundamental role in infection has been demonstrated by, among other things, the fact that the pharmacological block of L-type calcium channels slows the replication velocity of the Porcine Deltacoronavirus [15].
Intercellular free calcium is needed during the response to SARS, mediated by the complex of NOD-, LRR- and Pyrin Domain-containing protein 3 (NLRP3) inflammasome [16,17]. During a coronavirus infection, including SARS-CoV-2, calcium mediates the fusion of the viral envelope with the membrane of the host cell: the S1/S2 subunits of the spike fusion viral protein (S) interact in a calcium-dependent way with the endocytic protein machinery of the host cell and/or with the ACE2 (angiotensin-converting enzyme 2) transmembrane dominion, the designated receptor of SARS-CoV-2 expressed by the cells of the alveolar, intestinal and renal tubular epithelia, cardiomyocytes and endothelial cells [18,19].
A number of articles have been published supporting the hypothesis of a connection between vitamin D levels and risk of infection with SARS-CoV-2. The first of these studies, conducted in the U.S., analyzed 191,779 subjects in the three-month period from mid-March to mid-June 2020 and demonstrated a close correlation, even after adjusting for demographic factors (such as latitude, ethnicity, gender and age) [20].
A very recent systematic revision has found an inverse relationship between low temperatures, UV indices, cloud-free vitamin D UV doses (UVDVF) and prevalence of COVID-19 in Europe [21]. By contrast, one of our own studies (2021), conducted on 101,035 subjects in the greater Milan area, compared the pre-pandemic period (2019) with periods that included the so-called "first" (January-August 2020) and "second" (June-November 2020) waves. The results did not show any direct relationship between the indices of exposure to sunlight, 25(OH)D levels and infection with SARS-CoV-2. In addition, the study did not demonstrate any correspondence between 25(OH)D and domestic confinement during the lockdowns, while presupposing the existence of other variables which did not merit consideration [22].
Studies carried out on biobank statistics are also revealing: in 348,598 participants in the UK Biobank (ages 37-73), a correlation between 25(OH)D and risk of infection was lost after adjustments were made for confounding factors and ethnicity [23,24]. Another relevant factor, one which is often not reported in studies, is supplementation.
Given the great quantity (and variety) of studies on the topic, we must have recourse to meta-analyses. A large number of these point to an inverse association between vitamin D deficiency and risk of infection with SARS-CoV-2 (Tab. I). Some of these reports, however, highlight the close dependence of this association on other variables, such as advanced age, comorbidity (e.g., diabetes, hypertension and obesity) and in some cases male gender. For this reason, it is impossible to establish whether vitamin Deficiency represents a cause of increased risk of infection or rather whether it reflects (or is a result of) a physio pathological condition that in itself increases the risk of infection.

VITAMIN D AND SEVERITY OF COVID-19

The current scenario suggests a connection between hypovitaminosis D and the severity of COVID-19. Yet is equally evident the comorbidity and age play decidedly more significant roles. Nonetheless, chronic hypovitaminosis D can predispose patients to developing comorbidity and can for this reason have a more or less indirect determining effect on the severity of the illness: in fact, advanced age and obesity are connected both to the development of more severe COVID-19 and to hypovitaminosis D [36].
Some researchers have hypothesized that vitamin D plays a role in acute respiratory distress syndromes (ARDS). As we have seen, ACE2 functions as a binding site for the S viral protein: it is an enzyme which converts angiotensin II (Ang-II) into angiotensin 1-7 [Ang (1-7)]. The latter has a vasodilatory and anti-inflammatory action and protects against organ damage [37]. Following its bond with the S protein, the ACE2-virus particle complex is internalized, which therefore downregulates ACE2 enzymatic activity. The downregulation of ACE2 is associated with an abnormal inflammatory response which can cause tissue damage, which in turn leads to further downregulation of ACE2. This process can produce acute respiratory distress syndrome (ARDS) [38,39]. Vitamin D plays a protective role against ARDS, given its capacity to inhibit the expression of renin and the activity of the ACE/Ang-II/AT1R axis; on the other hand, it stimulates the ACE2/Ang-(1-7)/MasG axis (the Mas receptor associated with the G protein). For this reason, vitamin D acts as a negative endocrine modulator of the renin–angiotensin–aldosterone system (RAAS) [40,41]. Abnormal inflammatory response (cytokine storm) that results from infection with SARS-CoV-2 is in fact responsible for the development of COVID-19 and in some cases of manifestations of increased severity [42].
The so-called "cytokine storm," which is characterized by a massive, ongoing release of proinflammatory cytokines (IL-1, IL-6, TNFα, IFNγ), is responsible for the symptoms and organ damage (at the expense of the lungs and heart especially). Of these cytokines, IL-6 has been shown to be connected with prognoses and mortality in severe COVID-19 cases (circulating levels 2.9 times those recorded in less severe cases). The available data support the role of vitamin D in mitigating the cytokine storm by means of the induction of anti-inflammatory mediators (IL-10, IL-4, TGFβ). Furthermore, as we have seen, the induction on the part of 1,25(OH)2D in the expression of the phenotypes Th2 and T-reg – whose function is more strictly anti-inflammatory and regulating, at the expense of the proinflam-

TABLE I.
Summary of results from meta-analyses relative to association of three circulating levels of 25(OH)D with risk of infection with SARS-CoV-2.

No. included studies Study design	Patients	Association with risk of infection	Date of analysis	Ref.
8 CS, Re-Co	Age ≤ 18 Europe, North America	Yes (deficiency)	06-2021	25
13 Co, RCT	Average age 49-69 Asia, Australia, Europe, North America, South America	No (insufficiency, deficiency)	06-2021	26
72 CC, CS, Os, Pr-Co, Re-Co, RCT	/ Asia, Europe, North Africa, North America, South America	Yes	05-2021	27
49 Pr, Re	Average/median age 35-85 Asia, Europe, North America, South America, North Africa	Yes (serious deficiency, deficiency, insufficiency)	03-2021	28
43 CC, CS, Os, Pop, Pr, Pr-Co, Re, Re-CC, Reg	Median age 35-90 Asia, Europe, North Africa, North America	Yes (deficiency)	01-2021	29
21 CC, CS-Co	Average age 47-81 Asia, Europe, North America	Yes	12-2020	30
23 Re	Average/median age 35-77 Asia, Europe, North America	Yes (deficiency)	12-2020	31
14 CS, Pr-Os, Re-Os	Average/median age 46-81 /	Yes	12-2020	32
34 CC, Co, CS, RCT	Average age 42-88 Europe, Asia, North America	No	12-2020	33
14 CC, Co, CS	/ Asia, Europe, North America	Yes (deficiency)	12-2020	34
10 CC	Asia, Europe, North America	Yes (deficiency, insufficiency	9-2020	35

CC: case control study: Co: cohort study; CS: cross-sectional study; Os: observational study; Pop: population study; Pr: prospective study; RCT: randomized controlled trial; Re: retrospective study; Reg: population records.

matory Th1/Th17, which are especially involved in the cytokine storm – could play a role in mitigating the hyper-inflammatory response and, therefore, manifestations of COVID-19 [14].

An Iranian study based on data collected during the first wave (until May 2020) reports that 74% of patients hospitalized for COVID-19 were severely ill; of these, 32.8% had adequate vitamin D levels. Sufficient levels of vitamin D were associated with a less severe clinical condition, lower mortality rate, lower CRP levels and a relatively higher lymphocyte count. Only 9.7% of deceased patients over the age of 40 had adequate vitamin D levels, while 20% had levels < 30 ng/mL [43]. An Italian study from the same period examined 61 patients hospitalized for COVID-19, reporting that 72.1% had levels of 25(OH)D < 20 ng/mL (of whom 57.4% had levels that were even lower than 15 ng/mL). Levels of partial pressure of arterial oxygen and CRP as well as the severity of the pathology were correlated to vitamin D status [44].

Hypocalcemia resulting from hypovitaminosis D was also associated with more serious prognoses. Hypocalcemia was shown to be more frequent in males and elderly subjects, and calcium levels were inversely connected to CRP, LDH and the risk of hospitalization in intensive care units (ICUs). In addition, $[Ca^{2+}] < 2.00$ mmol/L at the time of admission was associated with more severe clinical conditions, organ damage, septic shock and mortality at 28 days. Concentration of serum calcium has in fact a prognostic value of 0.73, as defined by area below the AUC curve [1].

The findings of meta-analyses (Table II) show that while low vitamin D levels seem to be linked to a more severe symptomatology and greater risk of hospitalization, their association with other outcomes, in particular the risk of requiring mechanical ventilation, admission to intensive care and mortality, is less clear. In one of the most recent systematic reviews, which includes 20 studies and 12,806 patients between the ages of 42 and 81, no difference was found between subjects with deficient levels and those with normal ones with regard to mortality, admission to ICUs, recourse to ventilation and duration of hospitalization [45]. Similarly, an analysis of six studies and 1,424 patients did not show any difference in 25(OH) D levels between severe and non-severe COVID-19 patients nor any association with mortality [46].

The results of these studies show not only great variety and discrepancies but also different forms of bias. One example of bias is the temporal relation between vitamin D dosage and diagnosis of COVID-19, which in the various studies ranges from 12 months prior the diagnosis to simultaneous evaluation.

Regarding the utility of vitamin D supplementation, a meta-analysis of six RCTs and 551

TABLE II.
Summary of results of meta-analyses relative to association between circulating levels of 25(OH)D and clinical outcomes of COVID-19.

No. included studies Study design	Patients	Inverse association with outcome					Date of analysis	Ref.
		Severity/ Hospitalization	Duration of illness/ hospitalization	Mechanical ventilation	Transfer to ICU	Mortality		
13 Co, RCT	Ave. age 49-69 Asia, Australia, Europe, North America	/	/	/	No	No	06-2021	26
8 CS, Re-Co	Age ≤ 18 Europa, North America	Yes	/	/	/	/	06-2021	25
72 CC, CS, Os, Pr-Co, Re-Co, RCT	Asia, Europe, North Africa, North America, South America	Yes	/	/	/	Yes	05-2021	27
49 Pr, Re	Ave./median age 35-85 Asia, Europe, North America, South America, North Africa	Yes	/	/	Yes	Yes	03-2021	28
8 Po	/ Asia, Europe, North America	/	/	/	/	Yes	03-2021	47
43 Co, CS, Os, Os-CC, Pr, Re, Re-CC, Reg	Median age 35-90 Asia, Europe, North Africa, North America	Yes	/	/	/	Yes	01-2021	29
21 CC, CS	Average age 47-81 Asia, Europe, North America	Yes	/	/	/	No	12-2020	30
23 Re	Ave./median age 35-77 Asia, Europe, North America	Yes	/	/	/	No	12-2020	31
17 Os	/ Europe, Asia, Middle East North America	Yes	Yes	/	Yes	Yes	12-2020	48
14 CS, Pr-Os, Re-Os	Ave./median age 46-81 /	Yes	/	/	/	Yes (♂, diabetes, hypertension)	12-2020	32
34 CC, Co, CS, RCT	Average age 42-88 Europe, Asia, North America	No	No	No	No	No	12-2020	33

CC: studio caso-controllo; Co: studio di coorte; CS: studio *cross-sectional*; Os: studio osservazionale; Pop: studio di popolazione; Pr: studio prospettico; RCT: trial randomizzato controllato; Re: studio retrospettivo; Reg: registro di popolazione.

COVID-19 patients supports its effectiveness in terms of hospitalization in ICUs, mortality and positive PCR testing [49]. Similar results emerge from a meta-analysis of systematic reviews [50]. On the other hand, meta-analyses published as of June 2022 show that supplementation has limited effectiveness (Tab. III). In this case as well, the great variety of test designs makes it difficult to draw general conclusions.

From a physiological point of view, the vitamin D binding protein (VDBP) deserves attention. In addition to having high binding affinity to 1,25(OH)2D, it takes part in regulating the innate immune response and neutralizes free G-actin, which is released in great quantities following cellular death in ARDS. It further stimulates strong inflammatory response, intravascular coagulation, vesicular degranulation and leukocyte chemoattraction [51].

VITAMIN D AND VACCINE EFFECTIVENESS

The introduction of effective treatments in preventing severe forms of COVID-19, vaccines in particular, represented a turning point. Currently we do not possess any studies on the connection between vitamin D levels (including the effects of supplementation) and vaccine effectiveness. A positive correlation between 25(OH)D and antibody titer has been shown in a British study after eight weeks following a first dose of BNT162b2 [58], though not in a Greek sample following the second dose [59].

CONCLUSIONS

In spite of numerous observations, a cause-effect correlation between vitamin D status, risk of infection with SARS-CoV-2 and severity of COVID-19 has not been established. It is reasonable to suppose that sufficient vitamin D levels indicate balanced homeostasis, which in turn promotes an effective response to the

TABLE III.
Summary of results of meta-analyses relative to effects of vitamin D supplementation on risk of infections with SARS-CoV-2 and clinical outcomes of COVID-19.

No. incl. studies Study design	Patients and intervention	Inverse association with outcome						Date of analysis	Ref.
		Risk of infection	Severity/ hospitalization	Duration of illness/ hospitalization	Mechanical ventilation	Transfer to ICU	Mortality		
23 Co, Pr-Co, Re, Re- CC, Re-Co, RCT	Ages 15-103 Asia, Europe, North America, South America Primary prev. Secondary prev. Tertiary prev.	 No / /	 No n.d. /	 / / /	 / / /	 / / Yes	 / / Yes	01-2022	33
8 Os, RCT	Ave. age 53-88 Asia, Europe, South America	/	/	/	Yes	Yes	No	07-2021	52
2 RCT	Ave. age 49-69 Europe South America	/	/	/	/	No	No	06-2021	26
13 CC, Co, CS, Os, Pr, Re, RCT	Ave./ median age 45-90 Asia, Europe, South America Pre- and/or post-diagnosis supplementation	/	/	/	/	Yes	Yes	06-2021	53
4 CC, CS, EKO, Re-Co	Median age 49-74 Europe	/	No	/	/	/	No	06-2021	54
5 Os, RCT	Ave. age 53-88 Asia, Europe, South America Post-diagnosis supplementation	/	/	/	No	No	No	05-2021	55
10 Co, CS, Os, Pr-CS, Re, Re-CC, Re-Os, RCT	Ave. age 53-88 Europe, Asia, South America Post-diagnosis supplementation • High doses • Low doses	 / /	 / /	 / /	 / /	 No Yes	 No Yes	03-2021	56
3 Re-CC, RCT	Europe South America	/	/	/	/	Yes	No	12-2020	57

CC: case control study: Co: cohort study; CS: cross-sectional study; EKO: ecological study; Os: observational study; Pr: prospective study; RCT: randomized controlled trial; Re: retrospective study.

infection [14]. In support of this supposition, a recent systematic revision notes that a deficiency of micronutrients, including calcium and vitamin D, represents a relevant variable for risk of hospitalization in ICUs, intubation and death [60]. Other authors maintain that given the proven safety of vitamin D supplementation the mere possibility of a connection justifies the adoption of treatment protocols.
One element which has not been given due consideration but which deserves closer attention in terms of preventing future epidemics is the effect of chronic insufficiency or deficiency: this condition may represent a cause of – or at least a contributing factor to – dysfunctions at the base of the increased risk of adverse events. Such a hypothesis would be more plausible than what has been established so far.

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