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EDITORIAL

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Dear Readers,
In this issue, we return to the theme of a possible relation between vitamin D and the risk of infection or serious clinical symptoms from infection with SARS-CoV-2.
We propose this topic because many scientific publications continue to treat it (more than 80 over the last four months, as you can see from the selection of references following the two articles). The results of these treatments have sometimes been contradictory and do not by any means allow us to draw conclusions, much less certainties, one way or another. At the same time, some have taken the liberty of making assertions about the correlation in a way that to my mind is inappropriate and imprudent.
We are also focusing on this theme because COVID-19 continues to take a significant number of lives. Indeed, as we approach winter, there are still many unknowns regarding the public health and social impact of clinical manifestations of infection with SARS-CoV-2 variants. We therefore felt the need to provide an update and a rigorous and objective overview of the current state of scientific knowledge on this theme. As is the practice of this journal, this task has been entrusted to experts who have worked and published in the field.
The first article presents summaries of the rationale and evidence for as well as doubts about the possible role of vitamin D in conditioning the risk of infection with SARS-CoV-2 and the severity of COVID-19. The author rightly begins by summarizing current evidence on the physiological role of vitamin D in connection with innate immunity, in particular with regard to antimicrobial action, and with acquired immunity, in the context of modulating action which is primarily anti-inflammatory and promotes immune tolerance. He then looks at the evidence on the association between vitamin D levels and the specific risk of infection with SARS-CoV-2. In addition, he examines indirect evidence, such as the high prevalence of hypocalcemia in patients hospitalized for COVID-19. These cases are characterized by the possible expression of the dysregulation of calcium and phosphorus homeostasis caused by vitamin D deficiency or by reduced exposure to UVB rays, which especially affects vitamin D status, as is well known.
We should note that the author's own published findings have not brought to light any direct relationship between indices of exposure to sunlight (including confinement to homes during the lockdown), 25(OH)D serum levels and infection with SARS-CoV-2. At the same time, he does admit the possible existence of other variables which have not been taken into consideration. The author correctly highlights the strong dependence on co-variables of the association described in numerous studies between vitamin D and infection risk, such as old age, comorbidity, obesity, gender, ethnicity and supplementation (and we should note in passing that the last-named factor is often neglected). For this reason, vitamin D deficiency may not be the cause but the result, or simply a risk marker. The same interpretive doubts also characterize the numerous observations which currently report an inverse correlation between vitamin D levels and severity of COVID-19.
Nonetheless, our current state of knowledge does not allow us to exclude a possible co-responsibility of vitamin D deficiency in conditioning the seriousness of some clinical manifestations of the illness and its outcomes (hospitalization, recourse to mechanical ventilation, transfer to

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intensive care, and mortality). Some possible physio pathological mechanisms through which vitamin D deficiency might contribute to the pathogenesis of COVID-19 are believed to be known: vitamin D has the ability to mitigate the cytokine storm and acts as an endocrine modulator of the renin-angiotensin-aldosterone system, both of which are involved in the pathogenesis of acute respiratory distress syndrome. A response to current uncertainties might come from randomized controlled trials (RCTs) on vitamin D supplementation, as long as these are rationally designed and take into account that vitamin D could essentially act as a nutrient here and could therefore be effective only in patients with deficient levels. The meta-analysis of the few studies of this type which are currently available seem to indicate effectiveness on some outcomes, even if to a limited extent.

It is nonetheless undeniable that our present knowledge of the theme is characterized by broad variability and frequent discrepancy of the results. This circumstance indeed calls for a critical revision of the literature, which you will find in the second article of this issue. The authors summarize some of the most critical weaknesses of currently available publications, highlighting the use of unsatisfactory research tools or poorly designed trials. As the author of the first article recognizes, an important shortcoming is the bias of the temporal relation between vitamin D dosage and COVID-19 diagnosis, which varies in the different studies from one year before the diagnosis to simultaneous evaluation. This also appears relevant in light of the well-known phenomenon of reverse causality, that is, the fact that through inflammation the illness itself is associated with a reduction of 25(OH)D serum levels.

You will note that the statistical significance of correlations between 25(OH)D serum levels and the different outcomes depends on whether the dose was given before or during hospitalization. In addition, the reliability of currently available studies is not always sound, as several have been classified as low quality, given that they were subject to confounding factors or were lacking in detail or methodological adequacy. Then there is the problem of publication bias, which derives from the tendency to privilege the publication of studies with positive results. Other problems which sometimes characterize the literature on COVID-19 include the haste with which some preliminary data are confirmed, excessive simplification with generic conclusions which are not supported by statistically significant data, and the exceptional practice – given the urgency of the situation – of including observational studies in RCT meta-analyses. This has led to the publication of many low quality analyses with numerous confounding factors and therefore contradictory results. Such developments expose the scientific community to the risk of losing credibility.

All of these circumstances have contributed to the ongoing uncertainty as to the utility of vitamin D supplementation for the prevention and treatment of COVID-19. I personally fear that one day some people might conclude that in this field as well all we needed to do was rely on common sense to remedy a deficit, given the state of our knowledge about vitamin D and its degree of safety.

What do you think?

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Correlation between vitamin D deficiency and COVID-19: a critical review of the literature

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Just over two years since the World Health Organization (WHO) declared the SARS-CoV-2 outbreak a pandemic [1], we find on PubMed a surprising number of publications (256,087 articles as of June 19, 2022). A smaller but still significant number of these – 1,189 – regard "COVID-19 and vitamin D," a figure which amounts to an average of 1.5 publications daily. In comparison, 10,914 articles about "osteoporosis and vitamin D" have been published, although the first of these date to the beginning of the 1950's.

In fact, from the beginning of the outbreak interest in vitamin D has been intense. Toward the end of 2020, this journal published a summary of the first handful of studies available at that time, and in particular those that provided the first data on the association between vitamin D levels and risk of SARS-CoV-2 infection [2].

Since then, a tremendous number of articles have been published. In this article, we will summarize the observations obtained from a meta-analysis performed by Italian experts regarding the association between vitamin D status and clinical outcomes in patients with COVID-19 [3]. This meta-analysis is available on Open Access: we suggest that those interested in the topic read it in full. In this article we also offer a brief comment about the quality of the current evidence available on the benefits of vitamin D supplementation in these patients.

VITAMIN D STATUS AND CLINICAL OUTCOMES: MATERIALS AND METHODS

The primary endpoint of this analysis was to clarify the relationship between vitamin D status as a predictor of the severity of the disease, defined by the need for intensive care (IC) or mortality. The secondary endpoint, meanwhile, was to analyze the relationship between vitamin D status, susceptibility to SARS-CoV-2 infection and risk of hospitalization.

It is important note that because the disease itself is most likely associated with a reduction of 25-hydroxi-vitamin-D [25(OH)D] plasma levels [3], to overcome reverse causality bias (Fig. 1), the analysis separated those studies in which 25(OH)D values were measured before the infection (and which, therefore, were less influenced by this problem) from those in which 25(OH)D values were taken at the time of hospitalization.

Of 3,205 total studies that were initially identified, the selection reduced the sample to 54. As one would expect in a meta-analysis which includes observational studies, the quality of the selected works – which were assessed using the Newcastle-Ottawa scale, a specialized tool used to evaluate non-randomized studies – turned out to be quite heterogeneous, with several studies classified as low quality (scale score ≤ 6). As is well known, non-randomized studies are in fact subject to the influence of several confounding factors. Moreover, authors sometimes fail to adequately explain the methods with which studies are performed. Nonetheless, as the meta-analysis in question was limited to only studies of high quality (sensibility analysis), it has not given rise to specific concerns.

Another important aspect of a meta-analysis is the evaluation of the publication bias. This is a phenomenon that can be traced to today's world of scientific publishing, which tends to favor studies with "positive" (that is, statistically significant) results [4]. To contextualize and interpret results obtained from the analysis, then, it is essential to understand whether there is a significant risk of publication bias. This issue can be addressed by using specific tests, such as the Egger test and funnel plot inspection. Figure 2 shows two imaginary examples of funnel plots.

The meta-analysis in question revealed a certain degree of publication bias with regard to the outcome "transfer to ICUs" when the

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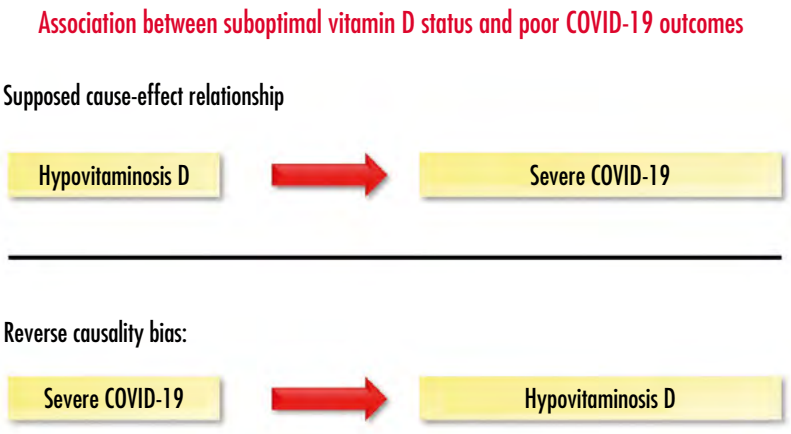

FIGURE 1.
Reverse causality (sometimes also called reverse causation). Bias in which dependent and independent variables are mistakenly confused.

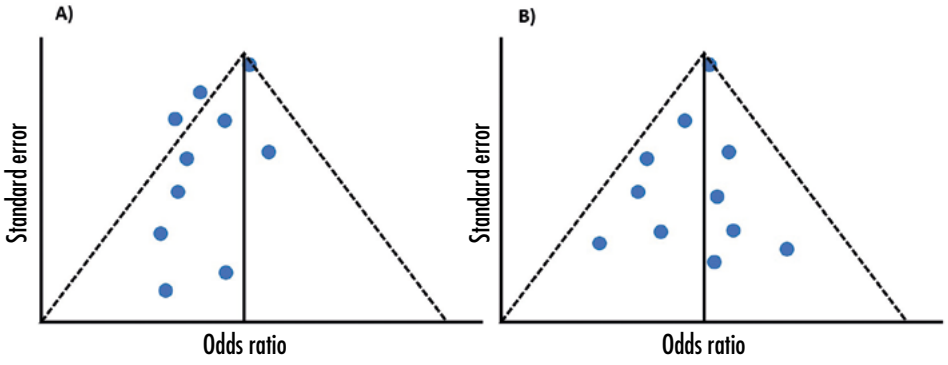

FIGURE 2.
Example of funnel plot in evaluating publication bias. Panel A: distribution of studies (each represented by a blue dot) is clearly asymmetrical, indicating probable publication bias. Panel B: no evident asymmetry; plot does not indicate publication bias.

threshold of plasma 25(OH)D < 75 nmol/L was used, and to that for mortality when the threshold of < 50 nmol/L was adopted.
On the basis of these observations, we cannot therefore exclude the possibility that results concerning these outcomes are, at least in part, overrated (even though additional analyses performed subsequently did not confirm this suspicion).

VITAMIN D STATUS AND CLINICAL OUTCOMES: RESULTS

Primary endpoint: transfer to IC and mortality

Probably the most important result of the meta-analysis was the observation of an increased risk for transfer to IC in patients with values of 25(OH)D < 25 nmol/L, both for dataset of all 11 analyzed studies (OR [odds ratio] 2.63; 95% CI [confidence interval] 1.45-4.77) and for studies in which 25(OH)D measurements were performed, for other reasons, before hospitalization (OR 2.55; 95% CI 1.28-5.08). As we have seen, this would exclude reverse causality because observation of low vitamin D levels in these studies preceded the development of the disease.
Similar results regarding an increased risk for transfer to IC were also found for the higher 25(OH)D thresholds (< 50 and < 75 nmol/L). However, these parameters lacked statistical significance in those studies in which 25(OH)D was measured before hospitalization.
Regarding the "mortality" outcome, the increased risk was confirmed for all the thresholds of 25(OH)D (for example: 25(OH)D < 25 nmol/L, 21 studies, mortality OR 2.60; 95% CI 1.93-3.49), but not in those studies in which vitamin D was measured before hospitalization.

Secondary endpoint: risk of SARS-CoV-2 infection and hospitalization

Increased risk of SARS-CoV-2 infection was confirmed for 25(OH)D levels lower than all the thresholds taken into consideration and also confirmed for pre-hospitalization levels < 25 nmol/L and < 50 nmol/L (4 studies, OR 1.42; 95% CI 1.09-1.84, and 3 studies, OR 1.35; 95% CI 1.08-1.69, respectively). A higher risk for hospitalization was also found for values lower than 75 nmol/L, but not when the analysis was limited to pre-hospitalization levels.
Finally, an increased OR was also found at all the thresholds for risk of hospitalization itself. However, increased risk for hospitalization was significant only for the threshold < 25 nmol/L in those studies in which vitamin D was measured before hospitalization (2 studies, OR 1.99; 95% CI 1.02-3.89).

VITAMIN D STATUS AND CLINICAL OUTCOMES: WHAT WE CAN CONCLUDE

With regard to COVID-19 the meta-analysis again noticed a close correlation between vitamin D insufficiency, risk of developing the disease and poor clinical outcomes. As we have already mentioned, although the authors tried to correct such biases as reverse causality, it is not in the nature of observational studies (from which this meta-analysis stems) to demonstrate a possible cause-effect relationship. Observational studies can bring to light a correlation between two variables, which does not necessarily imply a nexus between cause and effect. As is well known, establishing cause-effect relationships is the prerogative of randomized and controlled studies (RCTs). We will discuss these briefly below.
Nonetheless, the meticulous methodology which guided the meta-analysis enabled the authors to limit the effects of these biases and to produce an overview of currently available observational data. These findings suggest that vitamin D deficiency represents an indicator of risk for SARS-CoV-2 infection and a resulting unfavorable evolution.
In any case, as we already have extensively claimed in several editorials published in the pre-COVID-19 era, it is evident that vitamin D deficiency must be treated, in line

with quality medical (and ethical) practice, especially in at-risk populations.

VITAMIN D SUPPLEMENTATION AND INTERVENTION STUDIES: META-ANALYSIS AND BAD SCIENCE

As we have already emphasized, only data replication from rigorous RCTs can confirm the benefit of a specific intervention action. In fact, at the top of the hierarchy of evidence-based medicine, we find results of meta-analyses based (exclusively) on RCTs.

In specific cases, however, the *Cochrane Handbook* takes into account the possibility of including non-RCT data in a meta-analysis [6]. The first months of the pandemic, characterized by the urgent need to find safe and potentially effective treatments, may represent one of these exceptional cases.

At the same time, we need to remember that even though a pandemic can lower the bar that makes this trade-off acceptable, the uncertainty and the effect of confounding factors that can affect this kind of analysis are still multifarious and constant.

This pandemic took both doctors and researchers by surprise, and many egregious errors were made due to the hurry in endorsing some preliminary data, such as the case of proposed treatment with hydroxychloroquine and azithromycin. During the first half of 2020, observational data that were undoubtedly preliminary in nature indeed ended up affecting clinical practice on the part of many of us, before RCT results proved them proven to be off base [7]. In this case as well, the limitations of observational studies came to light. This is because it is often impossible to properly correct confounding factors (both context-sensitive and human), with the significant risk of producing efficient data which are both altered and overestimated [7].

I believe that we all wish to avoid repeating a similar situation in regard to vitamin D supplementation for COVID-19.

At present, there are only six RCTs on vitamin D supplementation and clinical outcomes [8-13] (Tab. I). In addition, in the majority of these studies the clinical outcome did not represent the primary endpoint; they were in fact not designed for this purpose. Of these six RCTs, only two (which in any case had significant methodological limitations) seem to indicate some degree of effectiveness.

A seventh study (Lakireddy et al.) was even withdrawn after publication because it was marred by serious shortcomings [14].

On the other hand, a cursory glance at PubMed reveals at least 10 systematic reviews with meta-analysis (which we won't treat here so as to not burden this article). Clearly, the majority of these meta-analyses also included observational studies, even if not exclusively. I believe that it is important to emphasize that this way of proceeding and this proliferation of qualitatively inadequate data puts the scientific community at risk of losing credibility. This is particularly true today, in light of the fact that we have treatments supported by RCTs and international recommendations [15].

For instance, one of these meta-analyses [16] (which can be defined as an umbrella meta-analysis because it in turn summarized seven systematic reviews with meta-analysis – all of observational studies) cites a reduction of as much as 50% in mortality due to COVID-19 in patients treated with vitamin D (OR 0.479; 95% CI 0.346-0.664). To better contextualize all of this, none of the treatments taken into consideration by the recommendations of the European Society of Clinical Microbiology and Infectious Diseases [15] has an effect size that comes anywhere near such a figure. One can readily understand that such result is lacking in credibility and represents a typical example of the saying "garbage in, garbage out." No matter how powerful and refined our method might be (in this case, the meta-analytic method), the result will be misleading, because if the quality of the data is poor the final output will be too.

To date, unfortunately, not even those who set out to perform a more selective analysis have demonstrated sufficient methodological rigor. Rawat et al. [17], for example, included only RCTs and "almost-experimental" studies (as they specified in the Materials and Methods section of their meta-analysis). In any case, classifying these studies as "almost-experimental" seems controversial, to say the least. Such trials were designed as simple observational studies and

TABLE I.
Summary table of currently available randomized controlled trials on treatment of COVID-19 with vitamin D.

Reference	Country	Sample number	Intervention and duration	Results
Sabico, Nutrients 2021	Saudi Arabia	69	5.000 *vs* 1.000 UI di D_3 for 2 weeks	Treated group showed faster recovery in terms of resolving coughing and ageusia
Murai, JAMA 2021	Brazil	240	200.000 UI D_3 (single dose) *vs* placebo	No significant differences in terms of hospital mortality, transfer to IC or need for mechanical ventilation
Castillo, J Steroid Biochem Mol Biol 2020	Spain	76	Calcifediolo 0,532 mg on days 1, 0,266 mg then weekly 3 and 7, until discharge from IC, *vs* placebo	Treated group had significant reduction of risk of transfer to IC
Maghbooli, Endocr Pract 2021	Iran	106	Calcifediolo 25 µg/die for 60 days *vs* placebo	No significant difference in clinical outcomes
Elamir, Bone 2022	Iran	50	Calcitriolo 0,5 µg/die for 2 weeks *vs* placebo	No significant difference in clinical outcomes; statistically significant reduction of use of oxygen in treated group
Cannata Andia, BMC Med 2022	Spain	543	Single bolus of 100,000 *vs* placebo	No significant difference in clinical outcomes

D_3: cholecalciferol; IC: intensive care: IU: international units.

have been indeed registered as such on clinicaltrials.gov.

Finally, I believe it is worth mentioning the systematic review with meta-analysis by Varikasuvu et al. [18], published in Expert Review of Anti-infective Therapy (a journal with an impact factor of greater than 5). The authors of this review, which includes only RCTs, demonstrate that COVID-19 patients who receive vitamin D supplementation have a lower degree of probability to be transferred to IC and lower chances of mortality and positive RT-PCR testing.

Nonetheless, upon closer examination of this paper we find elements which are not convincing. First of all, how should one interpret these conclusions? I expect that one would attribute significance to the fact that COVID-19 patients who receive vitamin D supplementation benefit in terms of fewer transfers to IC, a lower mortality rate and less frequent positive RT-PCR testing. However, the analysis of mortality, for example, does not provide a single significant result: OR 0.78; 95% CI 0.25-2.40.

We believe it is further important to note that some studies were used many times in the same analysis (for example, in the analysis of "severity", the same study was used both for the "mechanical ventilation" parameter and for that of "transfer to IC"). In addition, it is also important to note that a "significant" result (very ambiguous from our point of view) cited by the authors in the conclusion in fact referred to the overall analysis of all the outcomes taken together. In other words, a result was deemed "significant" (OR 0.6; 95% CI 0.4-0.92) by summing all the data pertaining to "COVID-19 severity", "RT-PCR positivity", "COVID-19 seropositivity" and "Deaths".

Although this fact was mentioned in the conclusions of the full-text article, we believe that for the sake of accuracy this should have been mentioned in the abstract, as well, the first part of the article to be read.

Finally, this study also unfortunately included the Lakireddy study, which, as we have seen, was retracted after publication. The bias risk evaluation of the above-mentioned meta-analysis (which can be consulted in the supplementary materials section) determined that it was of "some concern", a judgment that is nevertheless sufficient for its inclusion in the analysis. More specifically, the authors give it a positive assessment – "green light" – for the entry "randomization process."

CONCLUSIONS

Hypovitaminosis D is a widespread and problematic condition. In light of the great quantity of epidemiological studies which have brought to light a correlation with many pathological conditions [5], the use of inadequate research tools or poorly designed trials [5] has created much confusion among clinicians as to recommendations and modes of supplementation.

Something similar is taking place with regard to COVID-19. In this case the correlation between severe illness and hypovitaminosis D has been confirmed by solid data, while the question of whether supplementation confers real benefits once the pathology has developed is still open. My personal opinion is that it is our duty to demand that the quality of research in this field remain up to standard, so as to prevent further confusion created by studies which are compromised by evident methodological shortcomings.

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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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