VITAMIN D
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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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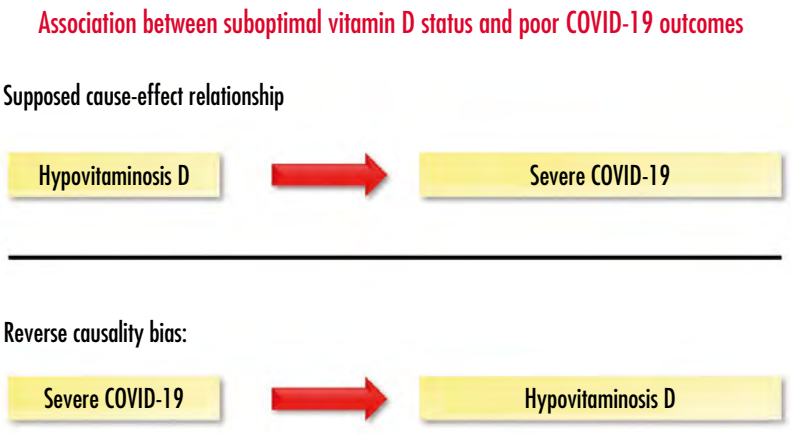

FIGURE 1.
Reverse causality (sometimes also called reverse causation). Bias in which dependent and independent variables are mistakenly confused.

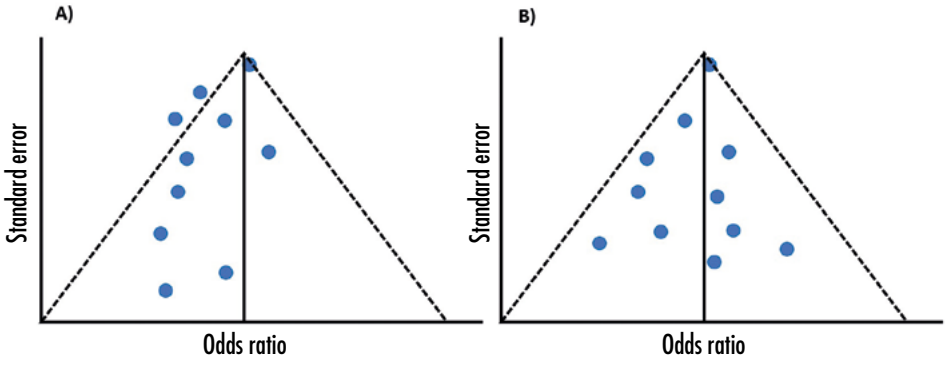

FIGURE 2.
Example of funnel plot in evaluating publication bias. Panel A: distribution of studies (each represented by a blue dot) is clearly asymmetrical, indicating probable publication bias. Panel B: no evident asymmetry; plot does not indicate publication bias.

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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