International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

The COVID-19 Pandemic, Seasons and the Vitamin D Laboratory Strategy

Darko Siuka[a], Teja Fabjan[b], Joško Osredkar[c]

[a]*Assist. Darko Siuka, MD, University Medical Centre Ljubljana, Division of Internal Medicine, Japljeva 2, Ljubljana, Slovenia; University of Ljubljana, Faculty of Medicine, Vrazov trg 2, Slovenia*

[b,c]*Teja Fabjan, University Medical Centre Ljubljana, Clinical Institute of Clinical Chemistry and Biochemistry, Zaloška cesta 2, 1000 Ljubljana, Slovenia; University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

[b,c]*Professor Joško Osredkar, PhD, University Medical Centre Ljubljana, Clinical Institute of Clinical Chemistry and Biochemistry, Zaloška cesta 2, 1000 Ljubljana, Slovenia;*

[c]*University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

[a]*Email: darko_siuka@yahoo.com,* [b]*Email: teja.fabjan@kclj.si,* [c]*Email: josko.osredkar@kclj.si*

Abstract

From the very beginning of the COVID-19 pandemic, the laboratory blood tests and markers are searched as a potential diagnostic tool for this disease and for disease severity assessment. Also from the beginning of the pandemic scientists published reports on the potentially important role of vitamin D on the course of the COVID-19 patients. According to the data there are seasonal fluctuations of COVID-19 severity, where the numbers of newly infected in summertime in contrast to wintertime, are the same or even higher, but there are much less death cases. The different seasonal incidence of infectious diseases has been well known since the Hippocratic period. Vitamin D is a molecule with potent immunomodulatory actions. The effectiveness against various upper respiratory infections is confirmed. Based on the findings of previous research on meta-analysis of interventional studies with vitamin D in respiratory viral infections, based on new COVID-19 observational studies and based on pilot randomized study where vitamin D in COVID-19 patients significantly reduced morbidity and alleviated the course of infection, we emphasize the importance of inclusion of vitamin D routine measurement, before vitamin D treatment in COVID patients becomes a routine.

---

* Corresponding author.

Vitamin D has a small chance of side effects in doses of prevention and therapy. Randomized double blinded clinical trials are needed to finally prove the benefit of supplementation of vitamin D in COVID-19 patients. Until then however, in the COVID pandemic, it is highly ethical to consider and use the current knowledge about the benefits of vitamin D substitution in patients with vitamin D deficiency or insufficiency

Keywords: Vitamin D; cholecalciferol; 25-OH-D_3; COVID-19; pandemic; SARS-CoV-2; seasons; summer; morbidity; mortality.

1. Introduction

The COVID-19 pandemic has strongly affected our health system, the global economy and values. In early spring 2020 in European countries, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) crisis began, with many severe cases and many deaths, especially in high-risk groups. Different countries have approached differently with preventive measures and strategies, which has resulted in different degrees of success. However, analyses were performed, where the effects on different disease courses and other reasons for the clinical outcome differences were questioned. From the very beginning of the COVID-19 pandemic, the laboratory blood tests and markers are searched as a potential diagnostic tool for this disease and for disease severity assessment. Some studies showed, that clinicians should consider the serum levels of CRP, ferritin, D-dimer, IL-6, cardiac troponin and lymphopenia for the severity and fatality prediction of COVID-19 hospitalized patients [1]. A more extensive list of potential markers is shown in Table 1. If some of this markers are altered, it was proposed, that the course of COVID-19 might be unfavourable.

Table1: Changes in laboratory parameters in patients with severe COVID-19 illness [2-5].

Blood count	Biochemistry	Inflammatory markers
↑ Leukocytes	↓ Albumin	↑ Erythrocyte sedimentation rate
↑ Neutrophils	↑ ALT	↑ CRP
↓ Lymphocytes	↑ AST	↑ Ferritin
↓ Thrombocytes	↑ Bilirubin, total	↑ Prokalcitonin
↓ Eosinophils	↑ Urea	↑ IL-2R
↓ Haemoglobin	↑ Creatinine	↑ IL-6
	↑ CK	↑ IL-8
Coagulation	↑ LDH	↑ IL-10
↑ PT	↑ Mioglobin	
↑ D-dimer	↑ CK-MB	
	↑ Troponin I	

1.1. Vitamin D and COVID-19 pandemic

Also from the beginning of the pandemic physicians of various specialties published scientific reports on the potentially important role of vitamin D on the course of the COVID-19 patients, the effect which may be similar

to reducing the risk of other viral infections, especially acute viral respiratory infections. The public awareness of vitamin D supplementation was risen during periods of respiratory infections and in the COVID-19 pandemic [6]. Retrospective studies were currently available confirming the association of low vitamin D levels with a more severe course of SARS-CoV-2 infection and poorer disease outcomes, and that vitamin D deficiency is a common characteristic of groups at higher risk for COVID-19 [7]. Even the latest, most extensive study, done by Merzon and al., on 14,000 individuals proves that in the case of vitamin D deficiency we can expect more severe infection and longer hospitalization [8]. Recently, results of an interventional randomized study of the benefit of vitamin D therapy in COVID-19 patients have been published. Of the 50 patients treated with 25 hydroxyvitamin D (25-OH-D_3), only one (2%) was admitted to the intensive care unit, none died, and all were discharged from the hospital without complications. Of the 26 patients not treated with vitamin D, as many as 13 patients (50%) required treatment in intensive care units, two died, and the rest were discharged without complications [9].

1.2. VITAMIN D laboratory measurement in the COVID-19 pandemic

There are two main reasons, why the laboratory measurement of 25-OH-D_3 should be routinely included in the laboratory protocol of the COVID-19 patients. First: According to the findings of respiratory infections previous research, meta-analysis, observational studies on COVID-19 and especially new randomised study supporting the evidence that vitamin D can greatly reduce the severity of COVID-19 disease, measuring of blood vitamin 25-OH-D_3 could predict severity and fatality of COVID patients. This means soon after SARS-Cov-2 infection confirmation, the serum vitamin D value could effectively predict hospital mortality of COVID-19 patients and vitamin D could be an early and helpful marker to improve management of COVID-19 patients. Second: There is new evidence of good results in preventing disease severity and reducing the need for treatment in intensive care units with vitamin D, but there is still no strategy for guidelines for inclusion of vitamin D therapy in COVID patient's treatment. It is known, that 25-OH-D_3 is the best vitamin D status biochemical marker. After we have the serum concentrations of total 25-OH-D_3, albumin and vitamin D-binding protein (DBP), we can calculate the free fraction of 25-OH-D_3, which is thought to be highly relevant for the local production and action of hormone, 1,25$(OH)_2D_3$ [10].

1.3. Vitamin D immunomodulatory actions and seasonal fluctuations

Vitamin D is a molecule with potent immunomodulatory actions. The effectiveness against various upper respiratory infections is confirmed. Vitamin D is known to be able to stop hyper inflammatory responses and actively accelerate the healing process of affected areas, especially in lung tissue. There are several reasons to encourage research into the action of vitamin D in patients with COVID-19. As no drugs are currently available for the treatment of COVID-19, the potential of vitamin D to alter the course of disease severity should be investigated [11]. According to the data there are seasonal fluctuations of COVID-19 severity, where the numbers of newly infected in summertime in contrast to wintertime, are the same or even higher, but there are much less death cases [12]. The different seasonal incidence of infectious diseases has been well known since the Hippocratic period. The reason for this is, among other things, that we are more active in certain parts of the year, which consequently means the variability of our immune system with concomitant changes in the

concentrations of vitamin D (25-dihydroxyvitamin D [25-OH-D3], melatonin and infectivity of pathogens. Of course, we must also take into account environmental factors, such as temperature, humidity, dehydration and UV light, which are most likely beneficial factors. Seasonal changes have also been shown to cause significant differences in the mutagenic responses of lymphocytes and consequently in the number of circulating lymphocytes, neutrophils, CD4 and CD8 cells, and IL-6. Furthermore, regulation of cytokines, namely IL-1β, IL-6, TNF-α, interferon (IFN)-γ, and IL-10 mediated by TLR-4 was observed intensely in the summer months compared to winter. The basis for this action of vitamin D is intracellular 1-α-hydroxylase, which is found in every cell. Two main modes of operation of vitamin D for immunity are described: increased immunity to antigens and modulation of the autoimmune response. The mechanism of increased antigen immunity was elegantly explained by Liu and his colleagues [13]. The antigen interacts with a cysteine-like receptor. The complex is introduced into the cell, where the genes for 1-α-hydroxylase and the vitamin D receptor (VDR) are activated. If sufficient 25-OH-D is present, the enzyme 1-α-hydroxylase converts it to 1,25-dihydroxy vitamin D ($1,25\text{-}(OH)_2D$). This active form of vitamin D interacts with VDR and stimulates the expression of cathelicidin (bactericidal peptide) and 24-hydroxylase, a catabolic enzyme that inactivates $1.25\text{-}(OH)_2D$ to complete the reaction [14,15]. As presented by Fares significant seasonal vitamin D level variations were observed in several communities, and the conversion rate of pro-vitamin D_3 to pre-vitamin D_3 in June and July is the highest, and then is gradually decreasing from August to October [16]. Regarding seasonal fluctuations, there is so much we can learn from the data from the Spanish flu pandemic from 1918/19. The second wave of the pandemic started in the beginning of the fall 1918 and was much more devastating than the first one. Grant also found in his research that there is an inverse relationship between UVB exposure and the mortality rate of influenza and the rate of pneumonia as a complication of influenza. He found significant correlations for the association of July UVB exposure with mortality rates ($r = -0.72$, $p = 0.009$) and rates of pneumonia as a complication of influenza ($r = -0.77$, $p = 0.005$). Similar results were also found for winter [17]. Historia magistra vitae est. Historically, before the advent of effective antibiotics, vitamin D (unknowingly) was used to treat infections such as tuberculosis by exposure to sunlight. Later, several cross-sectional studies were conducted linking lower vitamin D levels with increased infection. Vitamin D can modulate the innate and adaptive immune response, either by transcription of cathelicidins and beta defensin or by inhibiting T cell proliferation, resulting in a transition from the Th1 phenotype to Th2. In addition, it affects T cell maturation and facilitates the induction of T-regulatory cells, resulting in decreased production of inflammatory cytokines (IL-17, IL-21) and at the same time increased production of anti-inflammatory cytokines such as IL-10. However, this is not the only effect, vitamin D also affects monocytes and dendritic cells. It inhibits the production of inflammatory cytokines in monocytes (IL-1, IL-6, IL-8, IL-12 and TNFα) and can therefore alleviate the cytokine storm, which is one of the main causes of death in COVID-19 [18].

1.4. Vitamin D deficiency high risk groups

As it is previously known, vitamin D deficiency is common in people who develop Acute Respiratory Distress Syndrome and is known to be a pathogenic factor in sepsis and mortality of intensive care units [19]. A more severe course of infection and higher mortality are expected in patients who have the following risk factors: men over 65 years of age who have certain chronic diseases: high blood pressure, diabetes, cardiovascular disease, respiratory disease, and who smoke [20]. This groups mostly coincide with high risk groups for vitamin D

deficiency or insufficiency. The risks for vitamin D deficiency encompass obesity, elderly, lack of proper sun (UV-B) exposure, dark skin, smoking, living with air pollution and the presence of co-morbid diseases such as infection, cancer, chronic respiratory disease, osteoporosis, sarcopenia and diabetes mellitus [21]. A Frech quasi-experimental study showed that bolus vitamin D3 supplementation of 80 000IE during or just before COVID-19 was in nursing-home frail elderly residents associated with less severe COVID-19 and better survival rate. The "Intervention group" of 66 residents with COVID-19 recieved vitamin D and the 33 residents "Comparator group" didn't. 82.5% of participants in the Intervention group survived COVID-19, compared to only 44.4% in the Comparator group ($P = 0.023$). The full-adjusted hazard ratio for mortality according to vitamin D3 supplementation was $HR = 0.11$ [95%CI:0.03;0.48], $P = 0.003$. Intervention group had longer survival time than Comparator [22]. In the times, such as the COVID-19 pandemic it is challenge for evidence-based medicine. At the beginning of the pandemic there were no strong evidence data on the effects of vitamin D in SARS-CoV-2 infection to create guidelines. But there were reliable data in the scientific literature on the beneficial effects of cholecalciferol in alleviating viral respiratory infections, and new studies were emerging. The European Centre for Disease Prevention and Control encouraged health care professionals to take actions that were logical in their own right, based on previously known findings in related fields. Health authorities accepted this in a broadest way when beginning with the decision-making process. These factors also influenced the implementation of any proposed response measures. In spite of the fact that the decisions rarely could be evidence based, they should be evidence informed [6].

1.5. Vitamin D side effects

Vitamin D is a vitamin that has a small chance of side effects in doses of prevention and therapy, except in the case of parathyroid disease, hypercalcemia, sarcoidosis, tuberculosis and kidney stones [23].

1.6. COVID-19 and vitamin D supplementation strategy

Based on the findings of previous research on meta-analysis of interventional studies with vitamin D in respiratory viral infections, based on new COVID-19 observational studies and based on new pilot randomized study where vitamin D in COVID-19 patients significantly reduced morbidity and alleviated the course of infection, we emphasize the importance of inclusion of vitamin D routine measurement, before vitamin D treatment in COVID patients becomes a routine. Based on the evidence from causal inference analysis by Davies and al, it is strongly recommending the supplementation with vitamin D. Vitamin D prophylaxis offers a widely available, low-risk, highly-scalable, and cost-effective strategy to prevent local outbreaks and end the COVID-19 pandemic. Vitamin D prophylaxis can be implemented immediately in advance of vaccines and medications under development, and moreover, offers long-term added value for the general health status of the population [24]. Radujkovic A and his colleagues explored possible associations of vitamin D status with disease severity and survival. They studied 185 patients diagnosed with COVID-19. When adjusted for age, gender, and comorbidities, Vitamin D deficiency was associated with higher risk of need for invasive mechanical ventilation and/or death (HR 6.12, 95% CI 2.79–13.42, $p < 0.001$ and HR 14.73, 95% CI 4.16–52.19, $p < 0.001$, respectively) [25]. Baktash V and his colleagues in their study has demonstrated that patients over the age of 65 years presenting with symptoms consistent with COVID-19 are more likely to be vitamin D

deficient [26]. The findings of the study, presented by Meltzer O D and his colleagues give us the conclusion that vitamin D status has an important role in the course and outcome of COVID-19 infection [27]. Future studies should give answers to several questions, on which we don't have definitive answers. If vitamin D does reduce COVID-19 incidence, it is tempting to consider whether it might reduce COVID-19 transmission. Vitamin D strengthens innate immunity, so it might be expected to decrease COVID-19 infection and transmission [28]. Vitamin D also affects metabolism of zinc, which decreases replication of coronaviruses [29,30].

1.7. Conclusion

Our conclusion is that the mechanism of vitamin D supplementation is imperative to protect high risk individuals from the escalation of severe clinical course and to prevent deaths, but also to provide sustainability of the health system and maintaining the economy. All about COVID-19 is still "new", actually first studies appeared in journals in January this year. There is a number of preprint information about this topic and in a few months we can expect to get more information. Randomized double blinded clinical trials are needed to finally prove the benefit of supplementation of vitamin D in COVID-19 patients. Until we have the results of these studies, however, in the COVID pandemic, it is highly ethical to consider and use the current knowledge about the benefits of vitamin D substitution in infectious diseases patients with vitamin D deficiency or insufficiency.

References

[1]. Velavan TP, Meyer CG, Mild versus severe COVID-19: Laboratory markers, International Jofurnal of Infectious Diseases, Volume 95, June 2020, Pages 304-307. https://doi.org/10.1016/j.ijid.2020.04.061

[2]. Zhang J-j, Dong X, Cao Y-y, Yuan Y-d, Yang Y-b, Yan Y-q, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020 2020/02/19;n/a(n/a).

[3]. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet (London, England). 2020.

[4]. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China Life Sciences. 2020 2020/03/01;63(3):364-74

[5]. Brandon Michael H, Maria Helena Santos de O, Stefanie B, Mario P, Giuseppe L. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a metaanalysis. Clin Chem Lab Med. 2020(0):20200369

[6]. Siuka, D., Pfeifer, M., & Pinter, B. (2020). Vitamin D Supplementation During the COVID-19 Pandemic. Mayo Clinic proceedings, 95(8), 1804–1805. https://doi.org/10.1016/j.mayocp.2020.05.036

[7]. Benskin LL, A Basic Review of the Preliminary. Evidence That COVID-19 Risk and Severity Is Increased in Vitamin D Deficiency, Perspective, published: 10 September 2020, doi: 10.3389/fpubh.2020.00513

[8]. Merzon E, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, Frenkel-Morgenstern M.

Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. FEBS J. 2020 Jul 23:10.1111/febs.15495. doi: 10.1111/febs.15495. Epub ahead of print. PMID: 32700398; PMCID: PMC7404739

[9]. Entrenas Castillo M, Entrenas Costa LM, Vaquero Barrios JM, et al. "Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study" [published online ahead of print, 2020 Aug 29]. J Steroid Biochem Mol Biol. 2020;203:105751. doi:10.1016/j.jsbmb.2020.105751

[10]. Bikle D, Bouillon R, Thadhani R, Schoenmakers I. Vitamin D metabolites in captivity? Should we measure free or total 25(OH)D to assess vitamin D status? J Steroid Biochem Mol Biol. 2017 Oct;173:105-116. doi: 10.1016/j.jsbmb.2017.01.007. Epub 2017 Jan 16. PMID: 28093353.

[11]. Mohan M, Cherian JJ, Sharma A. Exploring links between vitamin D deficiency and COVID-19. PLoS Pathog. 2020 Sep 18;16(9):e1008874. doi: 10.1371/journal.ppat.1008874. PMID: 32946517.

[12]. Worldometer COVID-19 Coronavirus Pandemichttps://www.worldometers.info/coronavirus/ (accessed 17 July 2020).

[13]. Liu PT, Stenger S, Li H 14 et al.: Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770–1773 (2006)

[14]. Armas AG l. Vitamin D, infections and immune-mediated diseases. Int.J.Clin.Reumatol.2009; 4(1):89-103. doi: 10.2217/17584272.4.1.89

[15]. Heaney RP, Dowell MS, Hale CA, Bendich 4 A: Calcium absorptirron varies within the reference range for serum 25-hydroxyvitamin D. J. Am. Coll. Nutr. 22, 142 –146 (20 03)

[16]. Fares A. Factors influencing the seasonal patterns of infectious diseases. Int J Prev Med. 2013;4(2):128-132.

[17]. Grant WB, Giovannucci E. The possible roles of solar ultraviolet-B radiation and vitamin D in reducing case-fatality rates from the 1918-1919 influenza pandemic in the United States. Dermatoendocrinol. 2009;1(4):215-219. doi:10.4161/derm.1.4.9063

[18]. Aranow C. Vitamin D and the immune system. J Investig Med. 2011;59(6):881-886. doi:10.2310/JIM.0b013e31821b8755

[19]. Dancer RC, Parekh D, Lax S, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax. 2015;70(7):617-624. doi:10.1136/thoraxjnl-2014-206680

[20]. Zheng Z, M.D. et al, Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysisJ Infect. 2020 Apr 23. doi: 10.1016/j.jinf.2020.04.021

[21]. Kara M, Ekiz T, Ricci V, Kara Ö, Chang KV, Özçakar L. 'Scientific Strabismus' or two related pandemics: coronavirus disease and vitamin D deficiency [published online ahead of print, 2020 May 12]. Br J Nutr. 2020;1-6. doi:10.1017/S0007114520001749

[22]. Annweiler C, Hanotte B, de l'Eprevier CG, Sabatier JM, Lafaie L, Célarier T. Vitamin D and survival in COVID-19 patients: A quasi-experimental study. J Steroid Biochem Mol Biol. 2020 Oct 13:105771. doi: 10.1016/j.jsbmb.2020.105771. Epub ahead of print. PMID: 33065275; PMCID: PMC7553119. Marcinowska-Suchowierska E, Kupisz-Urbańska M, Łukaszkiewicz J, Płudowski P, Jones G. Vitamin D Toxicity-A Clinical Perspective. Front Endocrinol (Lausanne). 2018;9:550. Published 2018 Sep 20.

doi:10.3389/fendo.2018.00550

[23]. Davies G, Garami AR, Byers J. Evidence Supports a Causal Role for Vitamin D Status in Global COVID-19 Outcome. medRxiv preprint: doi.org/10.1101/2020.05.01.20087965

[24]. Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D Deficiency and Outcome of COVID-19 Patients. Nutrients 2020, 12, 2757; doi:10.3390/nu12092757

[25]. Baktash V, Hosack T, Patel N, et al. Postgrad Med J Epub ahead of print: doi:10.1136/ postgradmedj-2020- 138712

[26]. Meltzer O D, Best J T, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. JAMA Network Open. 2020;3(9):e2019722. doi:10.1001/jamanetworkopen.2020.19722

[27]. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. Nutrients. 2018;10 (11):1656. doi:10.3390/nu10111656

[28]. Greger JL. Effect of Variations in Dietary Protein, Phosphorus, Electrolytes and Vitamin D on Calcium and Zinc Metabolism: Nutrient Interactions. Marcel Dekker; 1988:205-228

[29]. Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog. 2010;6(11):e1001176. doi:10.1371/journal.ppat.1001176