Supplemental Online Content

Murai IH, Fernandes AL, Sales LP, Pinto AJ, Goessler KF, Duran CSC, Silva CBR, Franco AES, Macedo MB, Dalmolin HHH, Baggio J, Balbi GGM, Reis BZ, Antonangelo L, Caparbo VF, Gualano B, Pereira RMR. Effect of a Single High Dose of Vitamin D_3 on Hospital Length of Stay in Patients With Moderate to Severe COVID-19. *JAMA.* Published online February 17, 2021. doi:10.1001/jama.2020.26848

Supplement 1. Trial protocol and statistical analysis plan

This supplemental material has been provided by the authors to give readers additional information about their work.

Institutional Review Board Application

Vitamin D_3 Supplementation in Patients with COVID-19

Research project leaders: Rosa Maria Rodrigues Pereira, MD, PhD

Bruno Gualano, PhD

April 23, 2020

10

11

12

13

Table of Contents

1. Background

Vitamin D may enhance the innate[1-3] and adaptive immunity.[4, 5] It has been postulated that vitamin D supplementation could improve the function of macrophages and dendritic cells, thereby ameliorating overall immune response.[6] In fact, insufficient vitamin D status has been suggested as a potential risk factor for non-communicable[7] and acute respiratory tract diseases,[8, 9] including viral infections.[10]

Despite a growing body of evidence indicating the immunomodulatory role of vitamin D, the putative benefits of supplementary vitamin D_3 to patients with COVID-19 remain speculative and partially supported by limited data from observational studies and one small-scale, non-randomized clinical trial.[11-13]

In this context, our main *a priori* hypothesis was that a single dose of 200,000 IU of vitamin D_3 supplementation would increase 25-hydroxyvitamin D levels and shorten hospital length of stay among these patients.

2. Objectives

To investigate the safety and efficacy of an oral, single dose of vitamin D_3 supplementation (200,000 IU) compared to placebo on hospital length of stay and other clinical parameters in patients with severe COVID-19.

2.1 Primary endpoints

The assessment of hospital length of stay.

2.2 Secondary endpoints

The evaluation of mortality; number of patients admitted to the intensive care unit (ICU); number of patients who needed mechanical ventilation and duration of mechanical ventilation; serum levels of the following biochemical markers: 1) inflammatory markers (cytokines); 2) C-reactive protein; 3) 25-hydroxyvitamin D; 4) creatinine; 5) calcium; 6) physical activity levels.

3. Methods

3.1 Study design

This is a multicenter, double-blind, parallel-group, randomized placebo-controlled trial. Eligible patients will be randomized (1:1) into either vitamin D_3 group or placebo group. This study is registered in ClinicalTrials.gov as NCT04449718.

3.2 Screening and subjects recruitment

The screening and recruitment of patients will be conducted at Clinical Hospital of the School of Medicine of the University of Sao Paulo and Ibirapuera Field Hospital (included *a posteriori* via an addendum to the Ethics Committee).

3.3 Inclusion criteria

- Adults aged 18 years or older;
- Diagnosis of COVID-19 by either polymerase chain reaction (PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from nasopharyngeal swabs or

computed tomography scan findings (bilateral multifocal ground-glass opacities ≥ 50%) compatible with the disease;

- Diagnosis of flu syndrome with hospitalization criteria on hospital admission, presenting with respiratory rate ≥ 24 breaths per minute, saturation < 93% on room air or risk factors for complications, such as heart disease, diabetes mellitus, systemic arterial hypertension, neoplasms, immunosuppression, pulmonary tuberculosis, and obesity, followed by COVID-19 confirmation before randomization.

3.4 Exclusion criteria

- Patient unable to read and sign the written informed consent;
- Patient already admitted under invasive mechanical ventilation;
- Previous vitamin D_3 supplementation (> 1000 IU/day);
- Renal failure requiring dialysis or creatinine ≥ 2.0 mg/dL;
- Hypercalcemia defined by total calcium > 10.5 mg/dL;
- Pregnant or lactating women;
- Patients with expected hospital discharge in less than 24 hours.

3.5 Supplementation intervention

The vitamin D_3 supplementation group will receive an oral, single dose of 200,000 IU of vitamin D_3 dissolved in a 10 mL of peanut oil solution on the same day of randomization. This selected dose is within the recommended range for effectively promoting vitamin D sufficiency.[14] Patients in the placebo group will receive 10 mL of

peanut oil solution. The vitamin D_3 and placebo solutions were identical in color, taste, smell, consistency, and container.

3.6 Assessments

Hospital length of stay (primary endpoint), defined as the total number of days that patients remained hospitalized from the date of study admission until the date of hospital discharge or death.

Mortality; admission to ICU, defined as the number of patients admitted to the ICU; mechanical ventilation requirement, defined as the number of patients who needed mechanical ventilation; and duration of mechanical ventilation.

Serum levels of 25-hydroxyvitamin D will be assessed by a chemiluminescent immunoassay; serum levels of calcium, will be assessed by a NM-BAPTA method; serum levels of creatinine, will be assessed by a colorimetric assay based on kinetic Jaffe's reaction; and serum levels of C-reactive protein and D-dimer, both will be assessed by an immunoturbidimetric assay. The biochemical analyses were carried out in an accredited laboratory from Clinical Hospital.

4. Statistical analysis

4.1 Sample size calculation

Considering the lack of data available for sample size determination based on the primary outcome (i.e., hospital length of stay after vitamin D_3 supplementation in patients with severe COVID-19), the number of participants will be chosen on the basis of

feasibility, such as resources, capacity of research staff and facility, and available patients, in line with current recommendations.[15, 16]

4.2 Randomization

The randomization list will be created using a computer-generated code, which will be managed by a staff member who will have no role in the study.

4.3 Statistical Analysis

All analyses will be carried out following the intention-to-treat principle for all randomized patients, with no imputation for any missing data. Proportions will be compared between groups using χ^2 test and Fisher's exact test. Student's t-tests will be used for comparing continuous variables at baseline. The log-rank test will be used to compare the Kaplan-Meier estimate curves the number of days for hospital length of stay, the primary outcome. Cox regression models for hospital length of stay, admission to ICU and mechanical ventilation requirement will be used to adjusted by potential confounders in order to estimate hazard ratios (HR), with corresponding 2-sided 95% CI. Generalized estimating equations (GEE) for repeated measures will be used for testing possible differences in laboratory parameters, assuming group and time as fixed factors, with marginal distribution, and a first-order autoregressive correlation matrix to test the main and interaction effects. *Post-hoc* tests with Bonferroni's adjustment will be performed for multiple comparisons.

Statistical analyses will be performed with IBM-SPSS software, version 20.0. Significance level will be set at $\alpha = .05$.

5. References

1. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. Mar 2006;311(5768):1770-3. doi:10.1126/science.1123933
2. Aglipay M, Birken CS, Parkin PC, et al. Effect of High-Dose vs Standard-Dose Wintertime Vitamin D Supplementation on Viral Upper Respiratory Tract Infections in Young Healthy Children. *JAMA*. Jul 18 2017;318(3):245-254. doi:10.1001/jama.2017.8708
3. Campbell GR, Spector SA. Autophagy induction by vitamin D inhibits both Mycobacterium tuberculosis and human immunodeficiency virus type 1. *Autophagy*. Oct 2012;8(10):1523-5. doi:10.4161/auto.21154
4. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol*. Oct 2005;97(1-2):93-101. doi:10.1016/j.jsbmb.2005.06.002
5. Laplana M, Royo JL, Fibla J. Vitamin D Receptor polymorphisms and risk of enveloped virus infection: A meta-analysis. *Gene*. Dec 2018;678:384-394. doi:10.1016/j.gene.2018.08.017
6. Bilezikian JP, Bikle D, Hewison M, et al. MECHANISMS IN ENDOCRINOLOGY: Vitamin D and COVID-19. *Eur J Endocrinol*. Nov 2020;183(5):R133-R147. doi:10.1530/EJE-20-0665
7. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol*. Jan 2014;2(1):76-89. doi:10.1016/S2213-8587(13)70165-7
8. Aibana O, Huang CC, Aboud S, et al. Vitamin D status and risk of incident tuberculosis disease: A nested case-control study, systematic review, and individual-participant data meta-analysis. *PLoS Med*. 09 2019;16(9):e1002907. doi:10.1371/journal.pmed.1002907
9. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ*. Feb 2017;356:i6583. doi:10.1136/bmj.i6583
10. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. *PLoS One*. Jun 2010;5(6):e11088. doi:10.1371/journal.pone.0011088
11. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. *JAMA Netw Open*. 09 2020;3(9):e2019722. doi:10.1001/jamanetworkopen.2020.19722
12. Kaufman HW, Niles JK, Kroll MH, Bi C, Holick MF. SARS-CoV-2 positivity rates associated with circulating 25-hydroxyvitamin D levels. *PLoS One*. 2020;15(9):e0239252. doi:10.1371/journal.pone.0239252
13. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin Exp Res*. Jul 2020;32(7):1195-1198. doi:10.1007/s40520-020-01570-8
14. Kearns MD, Alvarez JA, Tangpricha V. Large, single-dose, oral vitamin D supplementation in adult populations: a systematic review. *Endocr Pract*. Apr 2014;20(4):341-51. doi:10.4158/EP13265.RA
15. Bacchetti P. Current sample size conventions: flaws, harms, and alternatives. *BMC Med*. Mar 2010;8:17. doi:10.1186/1741-7015-8-17
16. Bacchetti P, McCulloch CE, Segal MR. Simple, defensible sample sizes based on cost efficiency. *Biometrics*. Jun 2008;64(2):577-85; discussion 586-94. doi:10.1111/j.1541-0420.2008.01004_1.x