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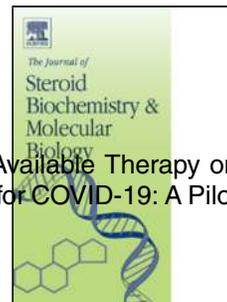
Marta Entrenas Castillo, Luis Manuel Entrenas Costa, José Manuel Vaquero Barrios, Juan Francisco Alcalá Díaz, José López Miranda, Roger Bouillon, José Manuel Quesada Gomez

"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"

Short title: Calcifediol Treatment in Hospitalized Covid-19 patients.

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Highlights

-The vitamin D endocrine system may have a variety of actions on cells and tissues involved in COVID-19 progression.
-Administration of calcifediol or 25-hydroxyvitamin D to hospitalized COVID-19 patients significantly reduced their need for Intensive Care United admission.
-Calcifediol seems to be able to reduce severity of the disease.

Abstract

Objective: The vitamin D endocrine system may have a variety of actions on cells and tissues involved in COVID-19 progression especially by decreasing the Acute Respiratory Distress Syndrome. Calcifediol can rapidly increase serum 25OHD concentration. We therefore evaluated the effect of calcifediol treatment, on Intensive Care Unit Admission and Mortality rate among Spanish patients hospitalized for COVID-19.

Design: parallel pilot randomized open label, double-masked clinical trial.
Setting: university hospital setting (Reina Sofia University Hospital, Córdoba Spain.)

Participants: 76 consecutive patients hospitalized with COVID-19 infection, clinical picture of acute respiratory infection, confirmed by a radiographic pattern of viral pneumonia and by a positive SARS-CoV-2 PCR with CURB65 severity scale (recommending hospital admission in case of total score > 1).

Procedures All hospitalized patients received as best available therapy the same standard care, (per hospital protocol), of a combination of hydroxychloroquine (400 mg every 12 hours on the first day, and 200 mg every 12 hours for the following 5 days), azithromycin (500 mg orally for 5 days. Eligible patients were allocated at a 2 calcifediol:1 no calcifediol ratio through electronic randomization on the day of admission to take oral calcifediol (0.532 mg), or not. Patients in the calcifediol treatment group continued with oral calcifediol (0.266 mg) on day 3 and 7, and then weekly until discharge or ICU admission. Outcomes of effectiveness included rate of ICU admission and deaths.

Results: Of 50 patients treated with calcifediol, one required admission to the ICU (2%), while of 26 untreated patients, 13 required admission (50%) p value X^2 Fischer test p<0.001. Univariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol treatment versus without Calcifediol treatment: 0.02 (95%CI 0.002-0.17). Multivariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol treatment vs Without Calcifediol treatment ICU (adjusting by Hypertension and T2DM): 0.03 (95%CI: 0.003-0.25). Of the patients treated with calcifediol, none died, and all were discharged, without complications. The 13 patients not treated with calcifediol, who were not admitted to the ICU, were discharged. Of the 13 patients admitted to the ICU, two died and the remaining 11 were discharged.

Conclusion: Our pilot study demonstrated that administration of a high dose of Calcifediol or 25-hydroxyvitamin D, a main metabolite of vitamin D endocrine system, significantly reduced the need for ICU treatment of patients requiring hospitalization due to proven COVID-19. Calcifediol seems to be able to reduce severity of the disease, but larger trials with groups properly matched will be required to show a definitive answer.

1.Introduction
A new coronavirus-induced pneumonia was called coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) on the February 11, 2020, at the same time the international virus classification commission announced that the new coronavirus was named coronavirus 2 severe acute respiratory syndrome (SARS-CoV-2) [1]. Its epidemic spread has increased since it appeared. On the 31st of January 2020, the WHO

announced that COVID-19 was labeled as Public Health Emergency of International Concern (PHEIC).

Patients with COVID-19 show clinical clusters of severe respiratory illness manifestations including fever, nonproductive cough, dyspnea, myalgia, fatigue, abnormal leukocyte counts, and radiographic evidence of pneumonia, which are similar to the symptoms of previous SARS-CoV and MERS-CoV infections [2].

SARS-CoV-2 infection can remain asymptomatic or cause modest symptoms. Severely sick patients require hospital admission and about 20 % of hospitalized patients will developed Acute Respiratory Distress Syndrome (ARDS) and require intensive care unit (ICU) treatment [3]. ARDS, also in patients with Coronavirus Disease 2019 (COVID-19) is a life-threatening condition [4][5]. Although frequencies vary according to series, more than 40 % of patients hospitalized because of COVID-19 pneumonia developed ARDS of which more than 50 % ultimately died [6]. ARDS onset is often rapidly progressive and appears approximately nine days after the onset of severe COVID-19 [2]. The epidemiologic, morbidity and mortality patterns of ARDS are similar regardless of the trigger [7]. Moreover, ARDS is a pivotal component in the development of multiple organ dysfunction and mortality risk [8]. In the absence well documented effective treatments [4], there is a strong interest in identifying a strategy [9] to taper down the severity of COVID-19, as it would reduce the morbidity and maybe mortality and lower the need for the limited ICU health care resources [10].

It has been proposed that the activation of the vitamin D receptor (VDR) signaling pathway may generate beneficial effects in ARDS [11] by decreasing the cytokine/chemokine storm, regulating the renin‑angiotensin system, modulating neutrophil activity and by maintaining the integrity of the pulmonary epithelial barrier, stimulating epithelial repair and tapering down the increased coagulability [12][13][14][15][16]. Recently, two ecological studies have reported inverse correlations between national estimates of vitamin D status and the incidence and mortality of IDOC-19 in European countries [17] [18]; lower concentrations of circulating 25 (OH) D have also been reported to be associated with susceptibility to SARS-CoV-2 infection [19] and the severity of the evolution of COVID-19 [20]. Vitamin D deficiency is frequent in wintertime even in Southern Spain [21] and even more so in patients requiring ICU treatment [22].

Therefore, considering the number of deaths associated to COVID-19, especially the speed with which ARDS is established in a significant number of patients, we performed a pilot study to assess the clinical effectiveness of treatment of patients hospitalized for COVID-19 with calcifediol (25-hydroxyvitamin D_3) in early stages to evaluate whether such treatment can reduce the need for admission to ICU and consequently the derived potential risk of death, as a preliminary step to a more extensive randomized clinical trial.

2. Methods

The study protocol was approved by the Pharmacy Committee, and by Ethics committee for the Treatment of COVID-19 of the Reina Sofía University Hospital, Córdoba, Spain EU. (Act-29/2020). The study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All patients and/or legal representatives were verbally informed about the objectives of the trial and their participation, by formally obtaining their consent, and its acceptance recorded in the electronic medical record of the Hospital.

2.1. Study Design Site and participants

Pilot Covidiol was a parallel pilot randomized open label, double-masked clinical study aiming to assess whether calcifediol can reduce the need for admission to ICU, and related death, as a previous part of the clinical trial Covidiol (Prevention and treatment with Calcifediol of Coronavirus induced acute respiratory syndrome (SARS) COVID-19 (COVIDIOL)" (NCT04366908)) and facilitate the sample calculation. This pilot trial was conducted at Reina Sofia University Hospital, Cordoba Spain.

Were included in the study seventy-sixth consecutive patients hospitalized with COVID-19 infection clinical [23][24] picture of acute respiratory infection, confirmed by a radiographic pattern of viral pneumonia and by a positive SARS-CoV-2 PCR with CURB65 severity scale (recommending hospital admission in case of total score > 1) [25]. Patients younger than 18 years and pregnant women were not included (Figure 1).

 All hospitalized patients received as best available therapy the same standard care, (per hospital protocol), of a combination of hydroxychloroquine (400 mg every 12 hours on the first day, and 200 mg every 12 hours for the following 5 days), azithromycin (500 mg orally for 5 days) and for patients with pneumonia and NEWS score≥5, a broad spectrum antibiotic (ceftriaxone2 g intravenously every 24 hours for 5 days) was added to hydroxychloroquine and azithromycin.

Hydroxychloroquine (EC50 = 0.72 µM) was chosen because it was in vitro more potent than chloroquine (EC50 = 5.47 µM). Based on physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models results, a loading dose of 400 mg twice daily of hydroxychloroquine sulfate given orally, followed by a maintenance dose of 200 mg given twice daily for 4 days is recommended for SARS-CoV-2 infection, as it reached 3 times the potency of chloroquine phosphate when given 500 mg twice daily 5 days in advance [26].

The patients were admitted to the ICU by applying the rigorous protocol of the Reina Sofia University Hospital (see supplementary material). Several fundamental aspects were considered when evaluating admission to the ICU: Presence of comorbidities, either individually or quantified in the modified age Charlson Comorbidity Index; Barthel's Index for functional assessment. It establishes the level of dependence of a patient according to his or her needs and clinical criteria: CURB-65 and SOFA scale and ATS/IDSA criteria [27]. A multidisciplinary Selection Committee was created, made up of intensivists,

pulmonologists, internists and members of the ethics committee who decided on admission to the ICU.

Sample Size Calculation was carried out for a pilot study with 75 patients randomized in the proportion of 2:1 to carry out the definitive trial (COVIDIOL) (NCT04366908). The sample size calculation is based on the proportion of a participant treated with Calcifediol could meet the criteria for admission to the Intensive Care Unit which is estimated as 5% (with 90% confidence intervals) and the proportion of a participant not treated with Calcifediol which could be 10%. According to these assumptions the estimated final sample size for our pilot clinical study was 50 patients in the arm of patients treated with Calcifediol and 25 patients in the group of patients not treated with Calcifediol [28]. The attrition rate is assumed to be 12%.

2.2. Procedures

Eligible patients were allocated at a 2 calcifediol:1 no calcifediol ratio through electronic randomization performed by hospital statisticians (figure 1) on the day of admission to take oral Calcifediol (Faes-Farma, Lejona, Spain), in soft capsules (0.532 mg), or not. Patients in the calcifediol treatment group continued with oral calcifediol (0.266 mg) on day 3 and 7, and then weekly until discharge or ICU admission [22][29]. Patients were followed-up until admission to ICU, hospital discharge or death.

Randomization and Masking

An electronically generated randomization 2:1 list was prepared by independent statisticians. The list was accessible only to nonmasked specialists in the study in an attempt to minimize observation bias. The patients' data were recorded in the hospital's electronic medical record, with blind access by the technical data collectors and the statistician who carried out the study.

Outcomes

Outcomes of effectiveness included rate of ICU admission and deaths. The working hypothesis of this pilot trial was that calcifediol treatment would decrease the need for ICU admissions and the potential risk of death associated with these admissions.

2.3. Laboratory Analysis and respiratory function test

Clinical samples for SARS-CoV-2 diagnostic testing were obtained according to WHO guidelines [30]. For each patient, a sampling strategy was implemented in which samples were obtained on admission. Upper respiratory tract samples were obtained by nasopharyngeal exudate sampling. Procedures for RNA extraction and real-time RT-PCR (rtRT-PCR) were undertaken in the local Central Microbiology Laboratory (Code 202 MagCore® Viral Nucleic Acid Extraction Kit and Allplex™ 2019-nCoV Assay by Seegene or VIASURE SARS-CoV-2 Real Time PCR Detection Kit).

Hematology analyses included blood count (Flow cytometry on ADVIA 2120i, Siemens Healthineers, Erlangen, Germany) and coagulation study including D-Dimer (clotting and immunoturbidimetric assay on ACL TOP 700, Instrumentation Laboratory/Werfen).

Biochemical tests including renal function, liver function, lactate dehydrogenase (spectrophotometric assay on Advia chemistry 2400 XPT, Siemens Healthineers, Erlangen, Germany), ferritin and C-reactive protein (immunoturbidimetric assay on Advia chemistry 2400 XPT, Siemens Healthineers, Erlangen, Germany. IL-6 (chemiluminescent immuno assay on Advia Centaur XPT, Siemens Healthineers, Erlangen, Germany)

Respiratory function was assessed by PaO2/FiO2 index [5]. A chest X-ray was taken in all patients on admission All X-ray tests were evaluated by an expert team of chest radiologist.

2.4. Statistical Analysis

Descriptive statistics were used for demographic, laboratory, and clinical prognostic factors related to COVID-19 for each treatment arm.

The comparison between groups of quantitative variables were performed by using t-test for qualitative variables, $\chi 2$ tests and Fisher exact tests (with frequencies <5) were used.

Univariate and multivariate logistic regressions were used to estimate Odds ratio and 95% CIs for the probability of admission to ICU. Significant p-value was considered when p<0.05.

All the analysis has been done using IBM SPSS Statistics software (SPSS).

The pilot trial was reported according to the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline [31].

3. Results

Table 1 shows demographic characteristics of the patients in both groups. Seventy-six patients (45 men (59%) and 31 women) were enrolled in the study and randomized: 26 without calcifediol treatment, 50 with calcifediol treatment (Figure 1). Mean age was 53±10 (mean± SD) years, being 54±9 years for men and 51±11 years for women. There was no significant gender difference in age between patients in each group (p=0.09).

Baseline factors associated with bad prognosis of COVID-19 are listed in Table 2 as absolute and relative frequencies for categorical variables, and as median plus standard deviation for numerical variables. In addition, both groups were compared for homogeneity at baseline.

At baseline, there was no significant difference in number of subjects with at least one risk factor. Patients assigned to calcifediol were slightly (not significantly) older, whereas the control group had a higher percentage of hypertension (Table 2).

Among 26 patients not treated with calcifediol, thirteen required ICU admission (50%), while out of fifty patients treated with calcifediol only one required admission to the ICU, whereas the other patients remained in conventional hospitalization

Although at baseline, there was no significant difference in number of subjects with at least one risk factor, the randomization did not achieve a homogeneous distribution of all the variables investigated between the two comparison groups (with and without calcifediol (Table2). A statistically significant difference was identified for the variable hypertension (26 had a history of hypertension of which 11 (42%) received Calcifediol and 15 (58%) not (CI: -0.58 - -0.13; p: 0.002) and close to statistical significance for diabetes 3 (6%) versus 5 (19%). Therefore, a multivariate logistic regression analysis was performed to adjust the model by possible confounding variables such as hypertension and type 2 diabetes mellitus for the probability of the admission to the Intensive Care Unit in patients with Calcifediol treatment vs Without Calcifediol treatment   (odds ratio: 0.03 (95%CI: 0.003-0.25) (Table 3). The dependent variable considered was the need to be treated or not in ICU (dichotomous variable).) CI:-0.30 - 0.03 p:0.08.

Of the patients treated with calcifediol, none died, and all were discharged, without complications. The 13 patients not treated with calcifediol, who were not admitted to the ICU, were discharged. Of the 13 patients admitted to the ICU, two died and the remaining 11 were discharged.

4. Discussion

In line with our hypothesis on a possible link between VDR activation and the severity of ARDS or COVID-19 [11], our pilot study suggests that administration of a high dose of calcifediol or 25-hydroxyvitamin D_3, a main metabolite of vitamin D endocrine system, significantly reduced the need for ICU treatment of patients requiring hospitalization due to proven COVID-19.

The best available treatment that at the beginning of the outbreak in our hospital, included the use of hydroxychloroquine/azithromycin therapy [23] [24] [26]. However, taking into consideration more recent data on the safety and efficacy of chloroquine and hydroxychloroquine in small randomized clinical trials, case series, and observational studies this treatment is no longer considered effective [32] in treating COVID-19.

Randomization generated groups with comparable percentage of unfavorable risk factors as there was no significant difference in subjects with at least one risk factor, except for high blood pressure and diabetes mellitus, known risk factors for unfavorable disease progression [2], which were more frequent in patients not treated with calcifediol.

However, even considering these factors, calcifediol significantly decreased the need for ICU admission in COVID-19 patients in a way not previously reported in this process until now [4]. From a mechanistic perspective there are good reasons to postulate that vitamin D endocrine system favorably modulates host responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), both in the later hyperinflammatory and early viraemic phases of COVID-19. as outlined in our previous review [11].

It is important to highlight that the cuboidal alveolar coating cells type II (ACII), like the cells of the immune system express all the enzymatic endowment (see above), to use

calcifediol as substrate synthesize 1.25 (OH)2D3 or calcitriol [33]. With high basal expression of 1α-hydroxylase activation and low expression of inactivating enzyme (24-hydroxylase).The result is that ACII constitutively convert calcifediol to 1,25-dihydroxyvitamin D3, the hormonal form of the endocrine system of vitamin D. The calcitriol generated by the ACII acting on themselves and cells of the immune system may then lead to increased expression of genes with important innate immune functions (the antimicrobial cathelicidin peptide, defensins and the TLR co-receptor CD14 etc...). In addition, in a viral infection model, dsRNA leads to increased regulation of 1α-hydroxylase and synergizes with calcifediol and calcitriol sequentially to induce cathelicidin [34].

It should be noted that the role of calcifediol and calcitriol in the animal model and ACII cells [12][34] and the immune system [35][36] were about equipotent suggesting that ACII cells actively converted calcifediol. Interestingly, when ACII cells are treated with a concentration of calcifediol ≥ 10 -7M (or ≥40 ng / ml) the same effects are achieved as when calcitriol is used, which is a guide to the serum levels of 25OHD3 to be achieved in our trial [12][34].

This pilot study has several limitations as it is not double-blind placebo controlled. On the other hand, in the first studies evaluating risk factors for severe disease and/or death from COVID-19, the possible role of obesity was not considered. Therefore, given the isolation characteristics of the patients, we did not collect the BMI, which would have allowed us to add obesity as a risk factor for severe evolution of COVID-19 [37] It is striking to consider that obesity shares with aging and black or asian ethnicity a surprising overlap as risk factors for severe COVID-19 and vitamin D deficiency [38].

Serum 25OHD concentrations at baseline or during treatment are not available [39][40]. Overall, adults living in the Córdoba area are relatively vitamin D deficient (16 ng/ml on average) in late winter and early spring [17]. Patients with severe ARDS [28][29] or requiring ICU [30] are [17] frequently severely vitamin D deficient. In addition, low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity [20].

Furthermore, to correct vitamin D deficiency in severely sick patients much higher doses of vitamin D than usual are needed. Our study does not include a comparison with cholecalciferol, the native vitamin D3 form and nutritional substrate for calcifediol, so that we cannot conclude that calcifediol is superior to vitamin D itself. Nevertheless, calcifediol may have some advantages over native vitamin D. It has a more reliable intestinal absorption (close to 100%) and can rapidly restore serum concentrations of 25OHD as it does not require hepatic 25-hydroxylation. This is especially relevant in clinical situations whereby rapid restoration of serum 25OHD is desirable and *CYP2R1* expression is compromised. Such impaired *CYP2R1* activity has been well demonstrated in several animal models [41] and has also been observed in patients with COPD or asthma [42]. In addition, calcifediol is more potent when compared to oral vitamin D3 [43]. In subjects with a deficient state of vitamin D, and administering physiological doses (up to 25 µg or 1000 IU daily, approximately 1 in 3 molecules of vitamin D appears as

25OHD; the efficacy of conversion is lower (about 1 in 10 molecules) when pharmacological doses of vitamin D/25OHD are used.[42]

The tissue effects of restoring the activation of the vitamin D receptor (VDR) signaling pathway may be due to circulating endocrine 1,25(OH)2D or, more likely, on the local conversion (para/autocrine) of 25OHD into the active hormone in pulmonary alveolar cells, immune cells or other potential target tissues [33].

5. Conclusions.

In conclusion, our pilot study demonstrated that administration of calcifediol may improve the clinical outcome of subjects requiring hospitalization for COVID-19. Whether that would also apply to patients with an earlier stage of the disease and whether baseline vitamin D status modifies these results is unknown. Therefore, a multicenter randomized controlled trial using calcifediol, properly matched (Prevention and Treatment With Calcifediol of COVID-19 Induced Acute Respiratory Syndrome (COVIDIOL)), in 15 Spanish hospitals, funded by "Clinical Research Program at COVID-19 "Progreso y Salud" Foundation and Foundation for Biomedical Research of Córdoba (FIBICO), Spain, (registered as NCT04366908 in NIH Trialnet database) will be carried out with the number of patients recalculated from the data provided by this study.

An interesting perspective of the new COVIDIOL trial with the recently available information, could be to evaluate calcifediol associated to dexamethasone or other corticoid vs. dexamethasone or other corticosteroid, since dexamethasone, which has potent anti-inflammatory actions, has recently been shown to reduce mortality in hospitalized patients on Covid-19 who are on respiratory assistance [44]; so that treatment guidelines have been updated to recommend the use of glucocorticoids (including dexametasone) [45], now proposed as the best available treatment in many hospitals around the world

REFERENCES:

[1] P. Zhou, X. Lou Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R. Di Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature. 579 (2020) 270–273. https://doi.org/10.1038/s41586-020-2012-7.

[2] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet. 395 (2020) 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

[3] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J. Xia, T. Yu, X. Zhang, L. Zhang, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet. 395 (2020) 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7.

[4] E. Fan, D. Brodie, A.S. Slutsky, Acute Respiratory Distress Syndrome, JAMA. 319 (2018) 698. https://doi.org/10.1001/jama.2017.21907.

[5] V.M. Ranieri, G.D. Rubenfeld, B.T. Thompson, N.D. Ferguson, E. Caldwell, E. Fan, L. Camporota, A.S. Slutsky, Acute respiratory distress syndrome: The Berlin definition, JAMA - J. Am. Med. Assoc. 307 (2012) 2526–2533. https://doi.org/10.1001/jama.2012.5669.

[6] C. Wu, X. Chen, Y. Cai, J. Xia, X. Zhou, S. Xu, H. Huang, L. Zhang, X. Zhou, C. Du, Y. Zhang, J. Song, S. Wang, Y. Chao, Z. Yang, J. Xu, X. Zhou, D. Chen, W. Xiong, L. Xu, F. Zhou, J. Jiang, C. Bai, J. Zheng, Y. Song, Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China, JAMA Intern. Med. (2020). https://doi.org/10.1001/jamainternmed.2020.0994.

[7] G. Bellani, J.G. Laffey, T. Pham, E. Fan, L. Brochard, A. Esteban, L. Gattinoni, F.M.P. Van Haren, A. Larsson, D.F. McAuley, M. Ranieri, G. Rubenfeld, B.T. Thompson, H. Wrigge, A.S. Slutsky, A. Pesenti, Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries, JAMA - J. Am. Med. Assoc. 315 (2016) 788–800. https://doi.org/10.1001/jama.2016.0291.

[8] Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, S. Liu, P. Zhao, H. Liu, L. Zhu, Y. Tai, C. Bai, T. Gao, J. Song, P. Xia, J. Dong, J. Zhao, F.S. Wang, Pathological findings of COVID-19 associated with acute respiratory distress syndrome, Lancet Respir. Med. 8 (2020) 420–422. https://doi.org/10.1016/S2213-2600(20)30076-X.

[9] H. Bauchner, P.B. Fontanarosa, Randomized Clinical Trials and COVID-19: Managing Expectations, JAMA - J. Am. Med. Assoc. (2020). https://doi.org/10.1001/jama.2020.8115.

[10] R. Li, C. Rivers, Q. Tan, M.B. Murray, E. Toner, M. Lipsitch, Estimated Demand for

US Hospital Inpatient and Intensive Care Unit Beds for Patients With COVID-19 Based on Comparisons With Wuhan and Guangzhou, China, JAMA Netw. Open. 3 (2020) e208297. https://doi.org/10.1001/jamanetworkopen.2020.8297.

[11] J.M. Quesada-Gomez, M. Entrenas-Castillo, R. Bouillon, Vitamin D receptor stimulation to reduce acute respiratory distress syndrome (ARDS) in patients with coronavirus SARS-CoV-2 infections: Revised Ms SBMB 2020_166, J. Steroid Biochem. Mol. Biol. 202 (2020). https://doi.org/10.1016/j.jsbmb.2020.105719.

[12] Y.Y. Shi, T.J. Liu, J.H. Fu, W. Xu, L.L. Wu, A.N. Hou, X.D. Xue, Vitamin D/VDR signaling attenuates lipopolysaccharide-induced acute lung injury by maintaining the integrity of the pulmonary epithelial barrier, Mol. Med. Rep. 13 (2016) 1186–1194. https://doi.org/10.3892/mmr.2015.4685.

[13] Y.Y. Shi, T.J. Liu, J.H. Fu, W. Xu, L.L. Wu, A.N. Hou, X.D. Xue, Vitamin D/VDR signaling attenuates lipopolysaccharide-induced acute lung injury by maintaining the integrity of the pulmonary epithelial barrier, Mol. Med. Rep. 13 (2016) 1186–1194. https://doi.org/10.3892/mmr.2015.4685.

[14] J. Kong, X. Zhu, Y. Shi, T. Liu, Y. Chen, I. Bhan, Q. Zhao, R. Thadhani, Y. Chun Li, VDR attenuates acute lung injury by blocking Ang-2-Tie-2 pathway and renin-angiotensin system, Mol. Endocrinol. 27 (2013) 2116–2125. https://doi.org/10.1210/me.2013-1146.

[15] S.X. Zheng, J.X. Yang, X. Hu, M. Li, Q. Wang, R.C.A. Dancer, D. Parekh, F. Gao-Smith, D.R. Thickett, S.W. Jin, Vitamin D attenuates lung injury via stimulating epithelial repair, reducing epithelial cell apoptosis and inhibits TGF-β induced epithelial to mesenchymal transition, Biochem. Pharmacol. 177 (2020). https://doi.org/10.1016/j.bcp.2020.113955.

[16] J.M. Martinez-Moreno, C. Herencia, A.M. De Oca, J.R. Muñoz-Castañeda, M.E. Rodríguez-Ortiz, J.M. Diáz-Tocados, E. Peralbo-Santaella, A. Camargo, A. Canalejo, M. Rodriguez, F. Velasco-Gimena, Y. Almaden, Vitamin D modulates tissue factor and protease-activated receptor 2 expression in vascular smooth muscle cells, FASEB J. 30 (2016) 1367–1376. https://doi.org/10.1096/fj.15-272872.

[17] E. Laird, J. Rhodes, R.A. Kenny, Vitamin D and inflammation: Potential implications for severity of Covid-19, Ir. Med. J. 113 (2020). https://pubmed.ncbi.nlm.nih.gov/32603576/ (accessed August 13, 2020).

[18] P.C. Ilie, S. Stefanescu, L. Smith, The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality, Aging Clin. Exp. Res. 32 (2020) 1195–1198. https://doi.org/10.1007/s40520-020-01570-8.

[19] A. D'avolio, V. Avataneo, A. Manca, J. Cusato, A. De Nicolò, R. Lucchini, F. Keller, M. Cantù, 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2, Nutrients. 12 (2020). https://doi.org/10.3390/nu12051359.

[20] G. Panagiotou, S.A. Tee, Y. Ihsan, W. Athar, G. Marchitelli, D. Kelly, C.S. Boot, N. Stock, J. Macfarlane, A.R. Martineau, G. Burns, R. Quinton, Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalised with COVID-19 are associated with greater disease severity, Clin. Endocrinol. (Oxf). (2020).

https://doi.org/10.1111/cen.14276.

[21]   J.M. Mata-Granados, M.D. Luque de Castro, J.M. Quesada Gomez, Inappropriate serum levels of retinol, α-tocopherol, 25 hydroxyvitamin D3 and 24,25 dihydroxyvitamin D3 levels in healthy Spanish adults: Simultaneous assessment by HPLC, Clin. Biochem. 41 (2008) 676–680. https://doi.org/10.1016/j.clinbiochem.2008.02.003.

[22]   J.M. Mata-Granados, J. Vargas-Vasserot, C. Ferreiro-Vera, M.D. Luque de Castro, R.G. Pavón, J.M. Quesada Gómez, Evaluation of vitamin D endocrine system (VDES) status and response to treatment of patients in intensive care units (ICUs) using an on-line SPE-LC-MS/MS method, J. Steroid Biochem. Mol. Biol. 121 (2010) 452–455. https://doi.org/10.1016/j.jsbmb.2010.03.078.

[23]   Gobierno de España, Ministerio de Sanidad, Consumo y Bienestar Social - Documentos técnicos para profesionales - Coronavirus, (2020). https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCo v-China/documentos.htm (accessed June 22, 2020).

[24]   Tratamientos disponibles sujetos a condiciones especiales de acceso para el manejo de la infección respiratoria por SARS-CoV-2 - Agencia Española de Medicamentos y Productos Sanitarios, (n.d.). https://www.aemps.gob.es/la-aemps/ultima-informacion-de-la-aemps-acerca-del-covid‐19/tratamientos-disponibles-para-el-manejo-de-la-infeccion-respiratoria-por-sars-cov-2/?lang=en (accessed June 22, 2020).

[25]   W.S. Lim, M.M. Van Der Eerden, R. Laing, W.G. Boersma, N. Karalus, G.I. Town, S.A. Lewis, J.T. Macfarlane, Defining community acquired pneumonia severity on presentation to hospital: An international derivation and validation study, Thorax. 58 (2003) 377–382. https://doi.org/10.1136/thorax.58.5.377.

[26]   In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) - PubMed, (n.d.). https://pubmed.ncbi.nlm.nih.gov/32150618/ (accessed June 23, 2020).

[27]   L.A. Mandell, R.G. Wunderink, A. Anzueto, J.G. Bartlett, G.D. Campbell, N.C. Dean, S.F. Dowell, T.M. File, D.M. Musher, M.S. Niederman, A. Torres, C.G. Whitney, Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults, Clin. Infect. Dis. 44 (2007) S27–S72. https://doi.org/10.1086/511159.

[28]   W. Viechtbauer, L. Smits, D. Kotz, L. Budé, M. Spigt, J. Serroyen, R. Crutzen, A simple formula for the calculation of sample size in pilot studies, J. Clin. Epidemiol. 68 (2015) 1375–1379. https://doi.org/10.1016/j.jclinepi.2015.04.014.

[29]   S. Russo, L. Carlucci, C. Cipriani, A. Ragno, S. Piemonte, R. Del Fiacco, J. Pepe, V. Fassino, S. Arima, E. Romagnoli, S. Minisola, Metabolic changes following 500 μg monthly administration of calcidiol: A study in normal females, Calcif. Tissue Int. 89 (2011) 252–257. https://doi.org/10.1007/s00223-011-9513-1.

[30]   Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human

cases, (n.d.). https://www.who.int/publications/i/item/10665-331501 (accessed June 23, 2020).

[31]   K.F. Schulz, D.G. Altman, D. Moher, CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials, BMC Med. 8 (2010). https://doi.org/10.1186/1741-7015-8-18.

[32]   J. Geleris, Y. Sun, J. Platt, J. Zucker, M. Baldwin, G. Hripcsak, A. Labella, D.K. Manson, C. Kubin, R.G. Barr, M.E. Sobieszczyk, N.W. Schluger, Observational study of hydroxychloroquine in hospitalized patients with COVID-19, N. Engl. J. Med. 382 (2020) 2411–2418. https://doi.org/10.1056/NEJMoa2012410.

[33]   R. Bouillon, C. Marcocci, G. Carmeliet, D. Bikle, J.H. White, B. Dawson-Hughes, P. Lips, C.F. Munns, M. Lazaretti-Castro, A. Giustina, J. Bilezikian, Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions, Endocr. Rev. 40 (2019) 1109–1151. https://doi.org/10.1210/er.2018-00126.

[34]   S. Hansdottir, M.M. Monick, S.L. Hinde, N. Lovan, D.C. Look, G.W. Hunninghake, Respiratory Epithelial Cells Convert Inactive Vitamin D to Its Active Form: Potential Effects on Host Defense, J. Immunol. 181 (2008) 7090–7099. https://doi.org/10.4049/jimmunol.181.10.7090.

[35]   A. Rafique, L. Rejnmark, L. Heickendorff, H.J. Møller, 25(OH)D 3 and 1.25(OH) 2 D 3 inhibits TNF-α expression in human monocyte derived macrophages, PLoS One. 14 (2019). https://doi.org/10.1371/journal.pone.0215383.

[36]   O. Andrukhov, O. Andrukhova, U. Hulan, Y. Tang, H.P. Bantleon, X. Rausch-Fan, Both 25-hydroxyvitamin-D3 and 1,25-dihydroxyvitamin- D3 reduces inflammatory response in human periodontal ligament cells, PLoS One. 9 (2014) e90301. https://doi.org/10.1371/journal.pone.0090301.

[37]   A. Simonnet, M. Chetboun, J. Poissy, V. Raverdy, J. Noulette, A. Duhamel, J. Labreuche, D. Mathieu, F. Pattou, M. Jourdain, R. Caizzo, M. Caplan, N. Cousin, T. Duburcq, A. Durand, A. El kalioubie, R. Favory, B. Garcia, P. Girardie, J. Goutay, M. Houard, E. Jaillette, N. Kostuj, G. Ledoux, D. Mathieu, A.S. Moreau, C. Niles, S. Nseir, T. Onimus, E. Parmentier, S. Préau, L. Robriquet, A. Rouze, S. Six, H. Verkindt, High Prevalence of Obesity in Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation, Obesity. 28 (2020) 1195–1199. https://doi.org/10.1002/oby.22831.

[38]   A.R. Martineau, N.G. Forouhi, Vitamin D for COVID-19: a case to answer?, Lancet. Diabetes Endocrinol. (2020). https://doi.org/10.1016/S2213-8587(20)30268-0.

[39]   R.P. Heaney, Guidelines for optimizing design and analysis of clinical studies of nutrient effects, Nutr. Rev. 72 (2014) 48–54. https://doi.org/10.1111/nure.12090.

[40]   W.B. Grant, B.J. Boucher, H.P. Bhattoa, H. Lahore, Why vitamin D clinical trials should be based on 25-hydroxyvitamin D concentrations, J. Steroid Biochem. Mol. Biol. 177 (2018) 266–269. https://doi.org/10.1016/j.jsbmb.2017.08.009.

[41]   R. Bouillon, D. Bikle, Vitamin D Metabolism Revised: Fall of Dogmas, J. Bone

Miner. Res. 34 (2019) 1985–1992. https://doi.org/10.1002/jbmr.3884.

[42]   D.A. Jolliffe, C. Stefanidis, Z. Wang, N.Z. Kermani, V. Dimitrov, J.H. White, J.E. McDonough, W. Janssens, P. Pfeffer, C.J. Griffiths, A. Bush, Y. Guo, S. Christenson, I.M. Adcock, K.F. Chung, K.E. Thummel, A.R. Martineau, Vitamin D Metabolism is Dysregulated in Asthma and Chronic Obstructive Pulmonary Disease, Am. J. Respir. Crit. Care Med. (2020). https://doi.org/10.1164/rccm.201909-1867oc.

[43]   J.M. Quesada-Gomez, R. Bouillon, Is calcifediol better than cholecalciferol for vitamin D supplementation?, Osteoporos. Int. 29 (2018) 1697–1711. https://doi.org/10.1007/s00198-018-4520-y.

[44]   RECOVERY Collaborative Group, P. Horby, W.S. Lim, J.R. Emberson, M. Mafham, J.L. Bell, L. Linsell, N. Staplin, C. Brightling, A. Ustianowski, E. Elmahi, B. Prudon, C. Green, T. Felton, D. Chadwick, K. Rege, C. Fegan, L.C. Chappell, S.N. Faust, T. Jaki, K. Jeffery, A. Montgomery, K. Rowan, E. Juszczak, J.K. Baillie, R. Haynes, M.J. Landray, Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report., N. Engl. J. Med. (2020). https://doi.org/10.1056/NEJMoa2021436.

[45]   Corticosteroids (including dexamethasone). NIH website. Updated July 17, 2020. Accessed August, 2020. https://www.covid19treatmentguidelines.nih.gov/dexamethasone/

Tables

Table 1. Demographic characteristics.   15

	Group receiving Calcifediol (n=50)	Group without Calcifediol (n=26)	IC 95%	P
Age (years)	53.14 +/- 10.77	52.77 +/- 9.35	-0.34 – 9.60	0.07
Males [n (%)]	27 (54%)	18 (69%)	-0.38 – 0.07	0.20
Females [n (%)]	23 (46%)	8 (31%)	-0.07 – 0.38	0.20
Male´s age (years)	56.30 +/ 8.29	52.13 +/- 10.05	-9.67 – 1.41	0.14
Female´s age (years)	49.43 +/- 12.28	54.13+/- 7.99	-4.87 – 14.25	0.32

Results are expressed as mean +/- Standard Deviation.

Table 2. Prognostic factors for COVID-19 at baseline

Poor prognosis risk factor	Group receiving Calcifediol (n=50)	Group without Calcifediol (n=26)	IC 95%	P
≥ 60 years	14 (28%)	5 (19.23%)	-0.11 – 0.28	0.40
Previous lung disease	4 (8%)	2 (7.69%)	´-0.12 – 0.13	0.96
Previous Chronic kidney disease	0	0	-	-
Previous Diabetes mellitus	3 (6%)	5 (19.23%)	-0.30 – 0.03	0.08
Previous High blood pressure	11 (24.19%)	15 (57.69%)	-0.58 – -0.13	0.002
Previous Cardiovascular disease	2 (4%)	1 (3.85%)	-0.09 – 0.09	0.97
Immunosuppressed & transplanted	6 (12%)	1 (3.85%)	-0.03 – 0.20	0.24
At least one prognostic bad risk factor*	24 (48%)	16 (61.54%)	-0.37 – 0,10	0.26
PaO2/FiO2 (mean +/-SD)	346.57 +/- 73.38	334.62 +/- 66.33	-22.29 – 46.19	0.49
C-reactive protein (mg/L) (mean +/-SD)	82.93 +/- 62.74	94.71 +/- 63.64	-42.15 – 18.59	0.44
LDH (U/L)(mean +/-SD)	308.12 +/- 83.83	345.81 +/- 108.57	-82.46 – 7.08	0.10
D-Dimer (ng/mL) (mean +/-SD)	650.92 +/- 405.61	1333.54 +/- 2570.50	-360.29 – 1725.53	0.19
Lymphocytes < 800/µL	10 (20%)	6 (23.08%)	-0.16 – 0.23	0.75
Ferritin (ng/mL) (mean +/-SD)	691.04 +/- 603.54	825.16 +/- 613.95	-166.31 – 434.55	0.36
IL-6 (22/48) (pg/mL) (mean +/-SD)	28.88 +/- 75.05	19.54 +/- 19.45	-41.88 – 23.19	0.41

SD: Standard Deviation.

* Patients with at least one of the following risk factors (age >60, previous lung disease, chronic kidney disease, diabetes mellitus, hypertension, cardiovascular disease or Immunosuppressed and transplanted patients).

Table 3.- Requirements for admission to the Intensive Care Unit, in patients hospitalized with COVID-19 (treated or not with calcifediol)

	Without Calcifediol Treatment (n=26)	With Calcifediol Treatment (n=50)	p value ($\chi2$) Fischer Test
Need for ICU			<0.001
Not requiring ICU, n (%)	13 (50)	49 (98)	
Requiring ICU, n (%)	13 (50)	1 (2)	

* Univariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol treatment vs Without Calcifediol treatment: 0.02 (95%CI 0.002-0.17).

** Multivariate Risk Estimate Odds Ratio for ICU in patients with Calcifediol treatment vs Without Calcifediol treatment ICU (adjusting by Hypertension and T2DM): 0.03 (95%CI: 0.003-0.25).

Figure 1.- Patients Flow Diagram.

Enrollment

Assessed for eligibility (n=76)

Excluded (n=0)

Randomized (n=76)

Allocation

Allocated to calcifediol treatment (n=50)
- Received allocated standard intervention (n=50)
Received calcifediol treatment
-

Allocated to no calcifediol treatment (n=26)
- Received allocated standard intervention (n=26)
Did not receive calcifediol treatment

Follow-Up

Lost to follow-up (n=0)

Discontinued intervention (n=0)

Lost to follow-up (n=0)

Discontinued intervention (n=0)

Analysis

Analysed (n=50)
- Excluded from analysis (n=0)

Analysed (n=26)
- Excluded from analysis (n=0)

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