Calcifediol treatment and COVID-19-related outcomes

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Summary

Background COVID-19 is a major health problem because of acute respiratory distress syndrome, saturation of intensive care units (ICU) and mortality.

Methods Our study aims to elucidate the effect of calcifediol [25(OH)D_3] treatment on ICU admission and mortality, in patients admitted to COVID-19 wards of Hospital del Mar, Barcelona, Spain. A total of 930 participants were included. Participants (n=551) were randomly assigned to calcifediol treatment (532 ug on day one and 266 ug on day 3, 7, 15, and 30) at the time of hospital admission or as controls (n=379).

Findings ICU assistance was required by 110 (11.8%) participants. Out of 551 patients treated with calcifediol at admission, 30 (5.4%) required ICU, compared to 80 out of 379 controls (21.1%; p<0.0001). Logistic regression of calcifediol treatment on ICU admission, adjusted by age, gender, linearized 25(OH)D levels at baseline, and comorbidities showed that treated patients had a reduced risk to require ICU (RR 0.18 [95% CI 0.11;0.29]). Baseline 25(OH)D levels inversely correlated with the risk of ICU admission (RR 0.53 [95% CI 0.35;0.80]).
Overall mortality was 10%. In the Intention-to-treat analysis, 36 (6.5%) out of 551 patients treated with calcifediol at admission died compared to 57 patients (15%) out of 379 controls (p=0.001). Adjusted results showed a reduced mortality for more of 60%. Higher baseline 25(OH)D levels were significantly associated with decreased mortality (RR 0.40 [95% CI 0.24;0.67]). Age and obesity were also predictors of mortality.

Interpretation In patients hospitalized with COVID-19, calcifediol treatment at the time of hospitalization significantly reduced ICU admission and mortality.

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Research in context

Evidence before this study

Adequate vitamin D status has emerged as a potentially preventing factor for SARS-CoV-2 infection, disease progression, and mortality. Recent studies have suggested that vitamin D supplementation might reduce the risk of influenza and SARS-CoV-2-related infections, requirement of critical care and death by decreasing pro-inflammatory cytokine production, thereby, diminishing the risk of developing a cytokine storm, which ultimately leads to acute respiratory distress syndrome (ARDS) development.

Vitamin D's protective effects are supported by studies that point to a role of the hormone in innate and acquired immunity modulation, autophagy induction, and synthesis of reactive oxygen intermediates. In addition, vitamin D is known to be involved in the induction of antimicrobial peptides in response to both viral and bacterial infections. From a clinical standpoint, a systematic review and meta-analysis from randomized controlled trials including more than 11,000 participants before the onset of COVID-19, showed protective effects of daily or weekly vitamin D administration on the risk of acute respiratory tract infection. In addition, many clinical data indicate that vitamin D deficiency, which is common among critically ill patients, is associated with longer hospital and ICU length of stay, lung and other organ injury, prolonged mechanical ventilation, and death.

Added value of this study

The Barna-COVIDIOL study is the first study to evaluate the effects of calcifediol supplementation on COVID-19-related mortality. It is also the largest study to assess the impact of calcifediol supplementation on ICU admission. It included 930 COVID-19 hospitalized patients, most of who were vitamin D deficient, who were randomized to receive a relatively high dose of calcifediol (=25OHD, vitamin D's main metabolite) or no treatment. Calcifediol was preferred to other available vitamin D metabolites because of its excellent intestinal absorption and rapid replenishment of 25OHD serum levels.

The main findings were that early calcifediol administration reduced the need for ICU admission (RR 0.18 [95% CI 0.11;0.29]), and most importantly, it significantly reduced the overall mortality risk by more than 60% (RR 0.36 [95% CI 0.19; 0.67]). No adverse events were reported.

Implications of all the available evidence

Vitamin D deficiency is common and even more so in COVID-19 patients compared to the general population. Rapid correction of such deficiency by calcifediol is easy, cheap, and appears as highly effective to control disease severity and avoid fatal outcomes in the setting of SARS-CoV-2 infection.

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the etiologic agent of Coronavirus disease-19 (COVID-19), a rapidly spreading infection that has caused a global pandemic since its initial identification in China in December 2019. The clinical spectrum of the disease ranges from asymptomatic or mild flu-like symptoms to severe respiratory illness and death[1]. Epidemiological studies have estimated that approximately 20% of infected patients sought medical attention, and 10% required hospitalization, thereby tremendously impacting healthcare services worldwide[2,3]. The burden of COVID-19 has particularly overwhelmed critical care, leading to saturated intensive care units (ICU) by patients with SARS-CoV-2-mediated acute respiratory distress syndrome (ARDS). Therefore, there is an enormous interest by the medical and scientific community to identify risk and protective factors associated with the development of COVID-19-related severe outcomes. At the beginning of the pandemic no effective treatments were available. Even today only four drugs are approved by the FDA: the antiviral agent remdesivir[4], dexamethasone[5], baricitinib in combination with remdesivir[6] and the neutralizing antibody bamlanivimab (LY-CoV555) [7].

Adequate vitamin D status has emerged as a potentially preventing factor for SARS-CoV-2 infection, disease progression, and mortality[8,9]. Many data indicate that vitamin D deficiency, which is common among critically ill patients, is associated with longer hospital and ICU length of stay, lung and other organ injury, prolonged mechanical ventilation, and death[10].

Considering the aforementioned data, we evaluated the effect of calcifediol treatment and baseline 25(OH)D serum levels on COVID-19-related severe outcomes: ICU admission and mortality rate among patients hospitalized for COVID-19 in a population-based study on patients admitted to COVID-19 wards in Hospital del Mar, Barcelona, Spain.

Methods

Study design

Clinical data from patients with COVID-19 symptoms and testing PCR positive, hospitalized randomly at one of the eight COVID-19 Units in Hospital del Mar (Barcelona, Spain), were collected from March 1st to May 31st 2020 in the Barna-COVIDIOL cohort–a prospective, observational, non-selected, clinical cohort study. Each COVID-ward from Hospital del Mar was assigned to one single Medical Unit during the first pandemic outbreak in Barcelona (Spain). The study protocol was approved by the ethics committee of Parc de Salut Mar (Exp number 2020/9287) and was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All patients were verbally informed about the treatment options. Verbal consent was registered in the hospital's electronic medical record system.

Participants

Patients with SARS-CoV-2 infection with chronic conditions and/or severe COVID-19 symptomatology (criteria listed in supplemental material) were enrolled at admission. They were followed from the first day of admission to their COVID ward up to date of medical discharge or death. Eligible participants

were patients aged 18 years or older who tested PCR positive for SARS-CoV-2. A total of 930 were included: 551 received calcifediol at admission to the hospital and 379 were not treated (control group). Fifty patients of the control group who required ICU also received calcifediol during ICU admission (same dosage and schedule as for patients treated at admission) at the discretion of the treating physicians and outside the original protocol.

Clinical samples for SARS-CoV-2 testing were obtained and analysed according to WHO guidelines [Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: https://www.who.int/publications/i/item/10665-331501 (Interim guidance 17th January 2020)]. All hospitalized patients received the same standard therapy, consisting in hydroxychloroquine 400 mg/24h first day and 200 mg/24h 4 days with azithromycin 500 mg/24h 3 days, plus ceftriaxone 1 or 2 g/24h 7 days when there was bacterial superinfection. Patients with severe or critical conditions of pulmonary inflammation or clinical suspicion of cytokine storm were additionally treated with dexamethasone bolus (20 mg/day x 4 days) according to hospital guidelines.

SARS-CoV-2 positive patients were allocated in 8 COVID-19 wards. Calcifediol was prescribed to all patients on 5 randomly selected wards whereas no such therapy was given to patients in the other 3 wards. Oral calcifediol treatment [25(OH)D3] (Hidroferol® Faes-Farma, Lejona, Spain), in soft capsules was administered as following: first dose of 2 capsules (266 micrograms/capsule) at baseline (day 0), a second dose of 1 capsule at day 3, and subsequent doses of 1 capsule at days 7, 15, and 30. The non-treated control group were patients who did not receive calcifediol at the time of hospitalization.

Outcomes

The outcomes of the study were admission into ICU (yes/no) and mortality (yes/no). The effect of calcifediol administration was studied in a prospective open randomized controlled trial. The consequences of baseline vitamin D status were studied in a subset of 752 patients for whom baseline serum 25(OH)D concentrations were measured.

Variables

Serum 25(OH)D levels

Serum levels of 25(OH)D were measured at baseline through competitive immunoluminometric direct assay with direct-coated magnetic microparticles (coefficient of variation<10%) (Elecsys 25(OH)D total II, model 07028148190; Cobas e801 system, Roche Diagnostics GmbH, Mannheim, Germany).

Baseline levels were linearized using log10 for multivariate regression analysis. Moreover, participants were classified according their 25(OH)D levels: patients with a serum concentration below 20ng/ml were considered to be vitamin D deficient, while those with ≥20 ng/mL were considered to be vitamin D replete[11],[12].

Risk factors and comorbidities

At the time of recruitment, several clinical variables and risk factors were registered, including: age, gender, ethnicity, high blood pressure (HBP), obesity (body mass index ≥30), dyslipidemia,

cardiovascular disease (CVD), chronic kidney disease (CKD), diabetes mellitus, respiratory-related diseases, previous or current cancer, persistent viral infections, and autoimmune diseases.

Statistical methods

Descriptive statistics were used for demographic, laboratory, and clinical prognostic factors related to COVID-19. Comparisons between groups for quantitative variables were performed by Intention to treat analysis using t-test or Krustal-Wallis test. Chi-square tests were used for qualitative variables. Survival analysis was performed by Kaplan-Meier estimation. Multivariate logistic regressions were used to estimate adjusted Risk Ratios (RR) and 95% CIs for the probability of admission to ICU or mortality. Baseline levels of 25(OH)D were linearized by applying the base 10 logarithm. Age, sex and COVID-19 risk factors (i.e. HBP, dyslipidemia, CVD, obesity, previous or current cancer, CKD, chronic infections, autoimmune conditions, chronic respiratory diseases and type II diabetes mellitus) were selected as confounders. Statistical analysis was done with R for Windows version 3.5.3 (foreign, logisticRR, ggpubr splines, survival, survminer and compareGroups packages) and SPSS Statistics version 22.0. P values lower than 0.05 were considered significant.

Results

Patient characteristics

A total of 930 participants were included in the Barna-COVIDIOL cohort. Of them, 551 patients were treated with calcifediol at admission and 379 were not treated. Fifty patients of this non-treated group started calcifediol during admission to ICU (Figure 1).

Patient characteristics, stratified by calcifediol treatment at admission (intention-to-treat analysis; ITT), are reported in Table 1. In the treated group, 53% were men whereas the 47% were women. In the non-treated group, 59.6% were men and 40.4% were women (p=0.045). Significant differences were found in baseline 25(OH)D levels between groups where the treated group had a median higher than the non-treated group (p<0.001). No other significant baseline differences were found in demographic characteristics, features and illness condition between groups.

A total of 752 patients in the cohort had baseline 25(OH)D levels measured, with a median [Q1;Q3] of 14 [8;24] ng/mL. Of them, 495 (65.8%) had 25(OH)D levels <20 ng/ml: 332 (55.2%) in the treated group and 163 (49.5%) in the untreated group.

Effects of calcifediol treatment and baseline 25(OH)D levels on ICU admission.

ICU assistance was required by 110 participants (11.8%). Out of 551 patients treated with calcifediol (at admission), 30 (5.4%) required ICU, while out of 379 patients non-treated with calcifediol at admission, 80 (21.1%) required ICU (p<0.0001) (Figure 2A). Patients admitted to the ICU had significantly lower baseline 25(OH)D levels compared to patients who remained in COVID wards (median [Q1;Q3]: ICU patients 11 [7.5;15.5]; No-ICU 15 [9;26] ng/ml, p<0.0001). Obesity was significantly more frequent in

ICU patients (Table S1). Logistic regression of calcifediol treatment on ICU admission, adjusted by age, gender, linearized 25(OH)D levels at baseline, and comorbidities showed that treated patients had an 82% reduced risk to require ICU (RR 0.18 [95% CI 0.11;0.29]) (Table 2). Baseline 25(OH)D levels inversely correlated with the risk of ICU admission (RR 0.53 [95% CI 0.35;0.80]). The same type of analysis was performed using categorized 25(OH)D levels (<20 ng/ml or ≥20 ng/ml) showing that patients with adequate baseline levels (≥20 ng/ml) had a decreased risk of ICU admission compared to patients with deficient 25(OH)D levels (RR 0.45 [95% CI 0.24;0.84]) (Table 2). Obesity was also significantly associated with ICU admission (RR 2.55 [95% CI 1.29;5.04]) (Table 2).

Effects of calcifediol treatment and baseline 25(OH)D levels on mortality

Out of 930 hospitalized patients, 93 died (10%). First, we calculated the mortality risk according to the original intention-to-treat analysis (ITT): out of 551 patients who received calcifediol at admission, 36 died (6.5%), whereas of the original control group (n=379), 57 (15%) died (p<0.001) (Figure 2B). Logistic regression analysis adjusted by 25(OH)D levels, age, gender, and comorbidities showed the calcifediol treatment reduced the mortality for more of 60% (RR 0.36 [95% CI 0.19; 0.67]) (Table 3). Secondly, the patients who received calcifediol at admission to the hospital (n=551) were combined with the ones starting with this drug when admitted to the ICU (n=50): of these 601 patients, 49 died (8.2%) versus 44 deaths (13.4%) in the 329 subjects who never received calcifediol (p=0.011) (Fig. S1). Logistic regression analysis adjusted by 25(OH)D levels, age, gender, and comorbidities showed a not statistically significant reduction of mortality risk in treated individuals (RR 0.64 [95% CI 0.34;1.18]) (Table S2). An additional analysis was performed excluding those 50 patients who started calcifediol treatment during ICU admission. Out of 551 patients treated with calcifediol, 36 (6.5%) died, while out of 329 patients of the never-treatment group, 44 (13.4%) died (p=0.001) (Fig. S2). Logistic regression analysis adjusted by 25(OH)D levels, age, gender, and comorbidities showed the calcifediol treatment was associated with decreased mortality (RR 0.48 [95% CI 0.24;0.95]) compared to non-treated patients (Table S3).

COVID-19 patients who died had significantly lower baseline 25(OH)D levels (9.5 ng/ml [6.5;15.5], compared to patients who survived (median [Q1;Q3 14 ng/ml [9;26], p<0.0001).

Adjusted results showed that baseline 25(OH)D levels were significantly associated with mortality as adequate 25(OH)D levels reduced the mortality risk in about 60%. Age and obesity were identified as risk factors for mortality (p<0.01) (Table 3).

As a subanalysis, we evaluated the mortality of ICU admitted patients (n=110): The mortality rate of such patients who received calcifediol at time of hospitalization (n=30) was 20% versus 26% of mortality in subjects who started calcifediol after ICU admission (n=50) and 30% in patients never treated with calcifediol (n=30). Non-significant differences were found between groups.

Discussion

In this open randomized study conducted during the first European outbreak of the deadly COVID-19 pandemic, we have observed that, in hospitalized COVID-19 patients, treatment with calcifediol reduced the requirement for critical care by more than 80%. This supports the conclusion of a prior pilot trial in Cordoba in which calcifediol treatment lead to a reduction of more than 50% of ICU admission in hospitalized COVID-19 patients[13]. Furthermore, calcifediol started at the time of hospitalization (intention-to-treat analysis) reduced mortality by more than 50%. Importantly, our results indicate that early calcifediol administration, prior to ARDS development, is critical for mortality reduction, since initiation of calcifediol during ICU admission did not modify patient survival.

Calcifediol, or 25(OH)D3, was selected, rather than more commonly used cholecalciferol or native vitamin D3 itself, because of its excellent pharmacokinetic profile, including a high intestinal absorption (close to 100%), a Tmax of approximately 4 hours resulting in a rapid increase in serum 25OHD, and a half-life of 12-22 days[14]. Moreover, calcifediol does not require hepatic hydroxylation, which is frequently impaired in acutely ill patients, and thus more readably available for conversion to $1,25(OH)_2D$[14]. The study population had a relatively low vitamin D status as demonstrated by the mean serum 25OHD concentration at baseline (19+/-14.8 ng/ml). Whether the beneficial effects of calcifediol would also be applicable in less vitamin D deficient populations will require appropriate intervention studies.

The analysis of the Barna-COVIDIOL prospective cohort also showed that baseline serum 25(OH)D was significantly and inversely related to ICU requirement and mortality. Several observational studies have pointed to a relationship between vitamin D deficiency and COVID-19-related mortality and/or disease severity[15-18], including a large cross-sectional study performed across 20 European countries in which a negative correlation between vitamin D levels and mortality was observed[8]. Panagiotou et al. also reported a significantly greater prevalence of vitamin D deficiency in COVID-19 patients admitted to the ICU compared to those that not requiring critical care[19]. However, other studies failed to find an association between vitamin D and poor disease outcomes[19-22].

Recent publications have reviewed vitamin's D plausible immunomodulatory mechanisms of actions on SARS-CoV-2 infection which are likely to contribute to our study results[8,13]. Most likely, calcifediol interferes with COVID-19-induced ARDS development. Indeed, ARDS is the most common indication for admitting a COVID-19 patient into the ICU[23]. This life-threatening condition is the consequence of an inflammatory and diffuse alveolar injury of acute onset, which leads to bilateral lung infiltration and severe hypoxemia. The pathogenesis of ARDS is closely linked to an exacerbated pro-inflammatory cytokine response of the host[24], and it is precisely in this setting where vitamin D might exert its main beneficial effects. The adaptive immune system can be modulated by 1,25(OH)2D, most importantly by modifying the phenotype of dendritic cells (responsible for antigen presentation to T cells) leading to a decrease in pro-inflammatory T cells subtypes proliferation, while enhancing the production of regulatory T cells[25]. Pro-inflammatory cytokine release in macrophages is also decreased by 1,25(OH)2D[26].

Ultimately these effects are thought to curb the inflammatory cascade that leads to the cytokine and chemokine storm associated with the pathogenesis of ARDS.

Adequate vitamin D status could also play a role in preventing COVID-19 infection. It is well-documented that cells of both the innate and adaptive immune systems express the vitamin D receptor and CYP27B1, the latter responsible of converting 25OHD to 1,25(OH)2D[11]. Vitamin D signaling activates the expression of several genes that encode proteins involved in the innate immune response, such as NOD2 and CD14, which recognize pathogen-associated molecular patterns, antimicrobial peptides such as cathelicidin and human beta-defensin 2, and other immune-related signaling molecules[11]. From a clinical standpoint, vitamin D therapy was previously found to be able to reduce upper respiratory viral infections[27]. Of note, this was not tested in the present study which started calcifediol as secondary prevention.

The study also confirmed that age and obesity were additional risk factors. Indeed, obesity and age have been linked to COVID-19 severity and poor outcomes in multiple studies and at the same time are well-established risk factors for vitamin D deficiency[28]. Our analysis also included a logistic regression analysis eliminating the potential confounding effect of age and obesity.

There are several limitations in the present study. First, the study was not placebo controlled. However, as calcifediol was randomly administered, we can consider the effect of calcifediol as an open randomized trial. Second, serum 25OHD was not measured during follow-up. No dose response curve was tested, so that we cannot define the minimal required dose and there was also no comparison with the more commonly used vitamin D. However, considering the administered doses and the drug's pharmacokinetic profile, we assume it replenished 25(OH)D deposits in all treated patients.

In spite of its weaknesses, the study was adequately powered to detect possible effects on the essential hard end points of ICU admission and mortality.

In summary, calcifediol administered at hospitalization reduced the requirement for ICU admission and decreased mortality by more than 50%. Moreover, baseline 25(OH)D levels correlated negatively with ICU admission and mortality. These findings point to the relevance of an adequate vitamin D status as soon as possible in the setting of SARS-CoV2 infection. This is particularly important as vitamin D deficiency is frequent but easily correctable. Nonetheless, additional studies are necessary to elucidate the effects of circulating 25(OH)D levels and 25(OH)D3 treatment on COVID-19 disease severity in other populations with different baseline vitamin D status.

Declaration of interests

RB has small lecture or consultancy fees from Fresenius (Germany), Abiogen (Italy), Faes Farma (Spain) and Proctor & Gamble (Belgium). All other authors declare no competing interests.

Data sharing

Cohort database with the individual participant data will be shared after deidentification. Individual participant data will be available beginning 3 months and ending 1 year after publication. Supporting clinical documents, including the study protocol, statistical analysis plan, and the informed consent form, will be available immediately following publication for at least 1 year.

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References

1. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**(18): 1708-20.
2. Boban M. Novel coronavirus disease (COVID-19) update on epidemiology, pathogenicity, clinical course and treatments. *Int J Clin Pract* 2020: e13868.
3. Russo F, Pitter G, Da Re F, Tonon M, Avossa F, Bellio S FU, Gubian L, Monetti D, Saia M, Zanella F, Zorzi M, Narne E, Mantoan D. Epidemiology and public health response in early phase of COVID-19 pandemic, Veneto Region, Italy, 21 February to 2 April 2020. *Euro Surveill* 2020; **25**(47).
4. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med* 2020; **383**(19): 1813-26.
5. Group RC, Horby P, Lim WS, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. *N Engl J Med* 2020.
6. Kalil AC, Patterson TF, Mehta AK, et al. Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *N Engl J Med* 2020.
7. Nirula A, Shen L, Skovronsky DM. Neutralizing Antibody LY-CoV555 for Outpatient Covid-19. Reply. *N Engl J Med* 2020; **384**(2).
8. 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* 2020; **32**(7): 1195-8.
9. 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* 2020; **3**(9): e2019722.
10. Nair P, Venkatesh B, Center JR. Vitamin D deficiency and supplementation in critical illness-the known knowns and known unknowns. *Crit Care* 2018; **22**(1): 276.
11. Bouillon R, Marcocci C, Carmeliet G, et al. Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev* 2019; **40**(4): 1109-51.
12. Amrein K, Scherkl M, Hoffmann M. 25(OH)D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr* 2020; **74**: 1498–513.
13. 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". *J Steroid Biochem Mol Biol* 2020; **203**: 105751.

14. Quesada-Gomez JM, Bouillon R. Is calcifediol better than cholecalciferol for vitamin D supplementation? *Osteoporos Int* 2018; **29**(8): 1697-711.
15. Carpagnano GE, Di Lecce V, Quaranta VN, et al. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. *J Endocrinol Invest* 2020.
16. Maghbooli Z, Sahraian MA, Ebrahimi M, et al. Vitamin D sufficiency, a serum 25-hydroxyvitamin D at least 30 ng/mL reduced risk for adverse clinical outcomes in patients with COVID-19 infection. *PLoS One* 2020; **15**(9): e0239799.
17. 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**(9).
18. Ye K, Tang F, Liao X, et al. Does Serum Vitamin D Level Affect COVID-19 Infection and Its Severity?-A Case-Control Study. *J Am Coll Nutr* 2020: 1-8.
19. Panagiotou G, Tee SA, Ihsan Y, et al. Original publication: Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity. *Clin Endocrinol (Oxf)* 2020; **93**(5): 629-30.
20. Baktash V, Hosack T, Patel N, et al. Vitamin D status and outcomes for hospitalised older patients with COVID-19. *Postgrad Med J* 2020.
21. Hastie CE, Pell JP, Sattar N. Vitamin D and COVID-19 infection and mortality in UK Biobank. *Eur J Nutr* 2020.
22. Pizzini A, Aichner M, Sahanic S, et al. Impact of Vitamin D Deficiency on COVID-19-A Prospective Analysis from the CovILD Registry. *Nutrients* 2020; **12**(9).
23. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**(4): 420-2.
24. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol* 2017; **39**(5): 529-39.
25. Adorini L, Penna G. Dendritic cell tolerogenicity: a key mechanism in immunomodulation by vitamin D receptor agonists. *Hum Immunol* 2009; **70**(5): 345-52.
26. Helming L, Bose J, Ehrchen J, et al. 1alpha,25-Dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. *Blood* 2005; **106**(13): 4351-8.
27. 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* 2017; **356**: i6583.
28. Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. *BMJ* 2020; **368**: m1198.

Table 1: Patient characteristics stratified according to calcifediol treatment at admission

Calcifediol	Treated N=551 (59.2%)	Non-treated N=379 (40.7%)	P value
Mean age (years) ± (SD)	64.02 ± 15.8	62.04 ± 17.2	NS
Gender (% male)	292 (53%)	226 (59.6%)	0.045
Ethnicity (n (%)):			
White	468(84.9%)	332 (87.5%)	
Asian	44 (8.0%)	28 (7.4%)	NS
African American	6 (1.1%)	4 (1.1%)	
Latino	33 (6%)	15 (4.0%)	
Baseline levels of 25(OH)D (median ng/ml [Q1;Q3])	15 [9;28]	12 [8;19]	<0.001
HBP (n (%))	251 (45.6%)	173 (45.6%)	NS
Dyslipidemia (n (%))	158 (28.7%)	116 (30.6%)	NS
CAD (n (%))	109(19.8%)	71 (18.7%)	NS
Obesity (n (%))	41 (7.4%)	32 (8.4%)	NS
Cancer* (n (%))	49 (8.9%)	33 (8.7%)	NS
Chronic kidney disease (n (%))	47 (8.5%)	29 (7.7%)	NS
Chronic infections (n (%))	26 (4.7%)	16 (4.2%)	NS
Autoimmune conditions (n (%))	21 (3.8%)	9 (2.4%)	NS
Chronic respiratory diseases (n (%))	104 (18.9%)	81 (21.4%)	NS
Type II Diabetes Mellitus (n (%))	118 (21.4%)	71 (18.7%)	NS

Abbreviations: SD, standard deviation; Q, quartile; HBP, high blood pressure; CAD, cardiovascular disease; NS, Non-significant. * previous cancer or current

Table 2: Logistic regression analysis for the association of calcifediol treatment and 25(OH)D baseline serum levels with ICU requirement.

Using baseline 25(OH)D levels linearized (n=752)				
	RR	**Low CI**	**High CI**	**p value**
Calcifediol treatment	0.18	0.11	0.29	<0.001
Linear 25(OH)D	0.53	0.35	0.80	0.002
Obesity	2.80	1.41	5.54	0.003
Using baseline 25(OH)D levels categorized (n=752)				
	RR	**Low CI**	**High CI**	**p value**
Calcifediol treatment	0.18	0.11	0.30	<0.001
25(OH)D (>=20ng/ml)	0.45	0.24	0.84	0.013
Obesity	2.55	1.29	5.04	0.007
Adjusted by age, gender, 25(OH)D levels, and COVID-19 risk factors. **Abbreviations:** CI. 95% Confidence interval; Linear 25(OH)D. baseline values of 25(OH)D linearized by base 10 logarithm; Obesity. body mass index ≥ 30; RR. Risk Ratio; Calcifediol treatment: minimum of 2 capsules before ICU admission.				

Table 3: Logistic regression analysis for the association of calcifediol treatment at admission and 25(OH)D baseline serum levels with mortality. Original ITT analysis.

Using baseline 25(OH)D levels linearized (n=752)				
	RR	**Low CI**	**High CI**	**p value**
Calcifediol treatment	0.36	0.19	0.67	0.001
Linear 25(OH)D	0.40	0.24	0.66	<0.001
Age	1.10	1.07	1.13	<0.001
Obesity	4.22	1.71	10.4	0.002
Using baseline 25(OH)D levels categorized (n=752)				
	RR	**Low CI**	**High CI**	**p value**
Calcifediol treatment	0.36	0.19	0.66	0.001
25(OH)D (>=20ng/ml)	0.37	0.17	0.82	0.014
Age	1.10	1.07	1.13	<0.001
Obesity	3.69	1.49	9.12	0.005
Adjusted by age, Gender, 25(OH)D levels and COVID-19 risk factors. **Abbreviations:** CI. 95% Confidence interval; Linear 25(OH)D. baseline values of 25(OH)D linearized by base 10 logarithm; Obesity. body mass index ≥ 30; RR. Risk Ratio; Calcifediol treatment: minimum of 2 capsules before ICU admission.				

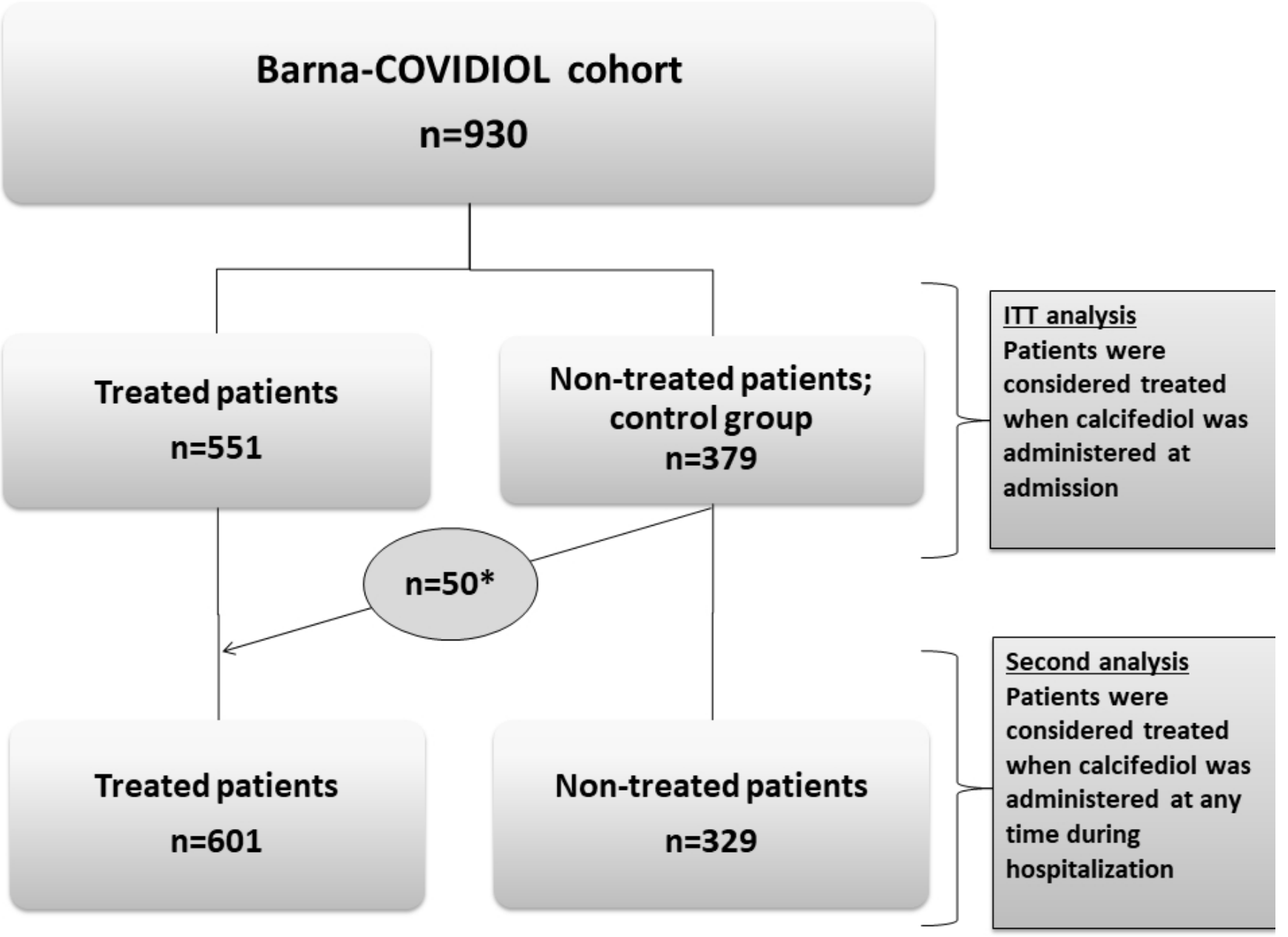
Figure legends

Figure 1. Flowchart showing the number of patient recruited in the Barna-COVIDIOL cohort allocated according to calcifediol treatment at admission-Intention-to-Treat (ITT) or during hospitalization. *50 patients from control group who started calcifediol during ICU admission.

Figure 2. Cumulative hazard plot of ICU requirement (A) and mortality (B) in Barna-COVIDIOL cohort according to calcifediol therapy. Graphs show Kaplan-Meier curves representing the outcomes of the study in terms of cumulative hazards. Differences were found between groups ($p<0.001$).

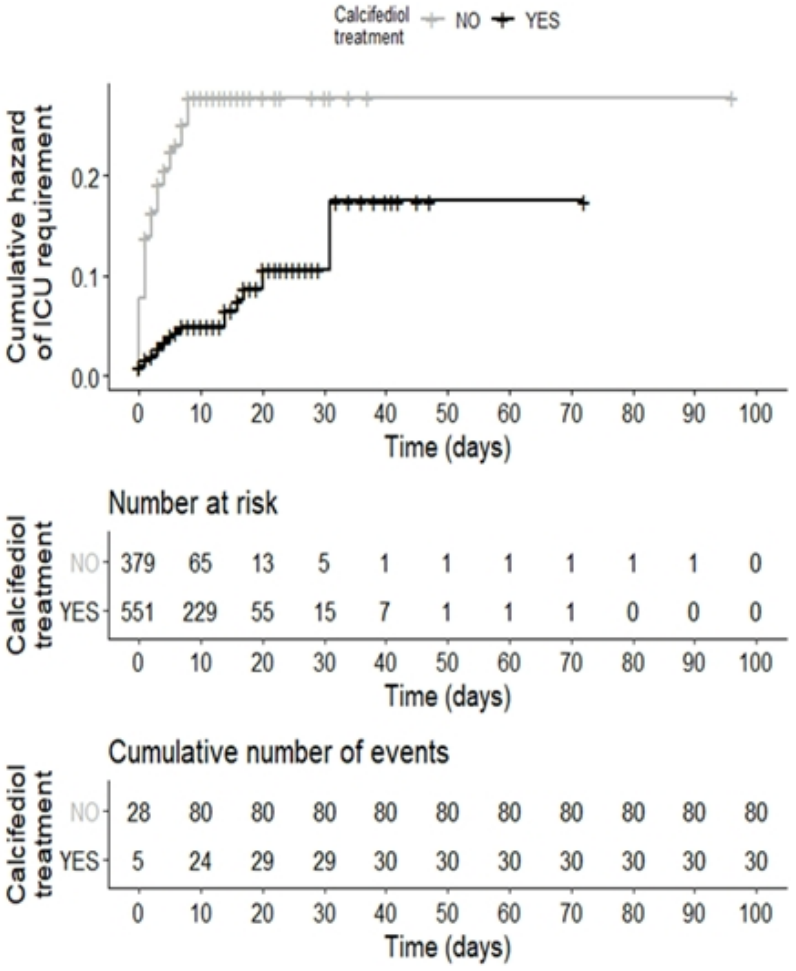
Figure S1. Cumulative hazard plot of mortality in Barna-COVIDIOL cohort according to calcifediol therapy during hospitalization. Graphs show Kaplan-Meier curves representing the outcomes of the study in terms of cumulative hazards. Differences were found between groups ($p<0.001$).

Figure S2. Cumulative hazard plot of mortality in Barna-COVIDIOL cohort according to calcifediol therapy at admission (excluding patients treated in ICU). Graphs show Kaplan-Meier curves representing the outcomes of the study in terms of cumulative hazards. Differences were found between groups ($p<0.001$).


Barna-COVIDIOL cohort
n=930
Treated patients
n=551
Non-treated patients; control group
n=379
n=50*
Treated patients
n=601
Non-treated patients
n=329
ITT analysis
Patients were considered treated when calcifediol was administered at admission
Second analysis
Patients were considered treated when calcifediol was administered at any time during hospitalization

A

Cumulative hazard plot of ICU requirement in Barna-COVIDIOL cohort according to calcifediol therapy at admission (ITT).



B

Cumulative hazard plot of mortality in Barna-COVIDIOL cohort according to calcifediol therapy at admission (ITT).