D.2.1.4 Hernandez 2020

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Study details

Study design	Case–control study
Study start date	10-Mar-2020
Study end date	20-May-2020
COVID-19 prevalence at the time of the study	Higher prevalence (e.g. during peak of first wave)
Aim of the study	The study aimed to assess the serum 25OHD levels in hospitalized patients with COVID-19 compared to population-based controls. In addition, it looked at the possible association between serum 25OHD concentrations and COVID-19 severity and mortality.
County/ Geographical location	Santander, Spain.
Study setting	Hospital for positive cohort; community for control cohort.
Population description	Patients with confirmed COVID-19 who were admitted to the University Hospital Marqués de Valdecilla made up the COVID-19 arm. They were recruited 10th - 30th March 2020. 19 patients were on vitamin D supplementation (11 taking cholecalciferol, 10 25,000 U monthly and 1 5,600 U weekly; 8 patients were taking calcifediol 0.266 mg monthly). Concerning immunomodulatory therapy, patients were selected for tocilizumab according to the institutional protocol. Thus, tocilizumab was indicated if there was clinical worsening with $PaO2/FIO^2$ ratio (PaFI) <300 and high serum acute-phase reactant levels when no contraindication for its use was present. Controls were taken from the Camargo cohort during their last follow-up visit in January-March 2020.
Inclusion criteria	COVID-19 arm: COVID-19 positive inpatients. Controls: participants who completed their last follow-up appointment in January-March 2020.
Exclusion criteria	Patients or controls with malabsorption disorders, liver cirrhosis, serum creatinine levels >1.9 mg/dl, or previous treatment with anticonvulsants. Controls who took vitamin D supplements for more than 3 months were also excluded. They were allowed in the COVID-19 positive arm but were analysed separately.

Vitamin D status measurements	Serum samples from Covid-19 patients were provided by the IDIVAL Biobank samples collection (internal code 2020-126). Serum 25OHD concentrations were determined in controls by a fully automated electrochemiluminescence system (Elecsys 2010, Roche Diagnostics, GmbH, Mannheim, Germany). The detection limit of serum 25OHD was 4 ng/ml. The intra-assay coefficient of variation (CV) was 5% and inter-assay was 7.5%. In COVID-19 patients, serum 25OHD levels were obtained at admission and assessed by automated competitive chemiluminescence assay (Liaison XL, DiaSorin Inc, Stillwater MN, USA). Our laboratory is DEQAS (Vitamin D External Quality Assessment Scheme) certified for this parameter. The detection limit of serum 25OHD was 4 ng/ml. The intra-assay and interassay CV were 2.58% and 7.83%, respectively.
Methods used to confirm COVID-19 infection	Qualitative detection of RNA from the SARS-CoV-2 was performed by using Real-Time PCR. Blood samples from the controls were obtained from an antecubital vein in the morning after a requested 12- hour overnight fast. The serum was divided into 0.5-ml aliquots and stored at -40ºC. Routine biochemical parameters were measured by standard automated methods in a Technicon Dax autoanalyzer (Technicon Instruments, CO. USA).
Methods for case-matching with control	Cases were sex-matched with population-based controls.
Methods of data analysis	4 models were used to compare 25(OH)D levels and associate 25(OH)D with clinical and laboratory parameters: 1) simple correlation between 25(OH)D and clinical indications; 2) comparing 25(OH)D levels between COVID-19 patients and controls that took into account confounding factors; 3) in COVID-19 patients only, simple association between 25(OH)D and disease severity; 4) in COVID-19 patients only, association between 25(OH)D and disease severity taking into account confounding factors. 1) Spearman rho was used to assess the relationships between serum 25(OH)D levels and several clinical and laboratory parameters. Serum 25(OH)D levels were stratified into four categories: below 10 ng/ml, between 10 and 20 ng/ml, between 20 and 30 ng/ml, and above 30 ng/ml. Vitamin D deficiency was defined as serum 25(OH)D levels <20 ng/ml (50 nmol/l). 2) A multivariable general linear model was set up to compare serum 25(OH)D levels between COVID-19 patients and controls (Bonferroni test), adjusting for confounding variables. 3+4) In the group of COVID-19 patients, univariable and multivariable binary logistic regression analyses were used to assess the association between vitamin D (as a continuous variable, or expressed as vitamin D deficiency or as quintiles) and the dependent variable of severity of the disease. Demographic, clinical, and outcome data of COVID-19 patients were gathered from the hospital records, stored in a computerized database, and independently reviewed by two researchers. Missing data were not imputed. Smoking status was coded as current or

	non-smoker. Immunosuppression included prolonged use (≥3 months) of corticoids (>10 mg/day of prednisone or equivalent) or immunomodulatory agents, and bone marrow or organ transplantation. Overall, the criteria for ICU admission were those of the guidelines by the American Thoracic Society and Infectious Diseases Society of America and the critical care ethic recommendations for the SARS-CoV-2 pandemic by the Intensive Medicine Spanish Society. The endpoint variable for COVID-19 severity has been defined as the composite of admission to the intensive care unit (ICU), requirement for mechanical ventilation, or in-hospital mortality. Clinical outcomes were monitored up to May 20, 2020. Continuous variables were expressed as mean +/- SD or median and interquartile range (IQR) and compared with the Student's t-test or Mann-Whitney U-test depending on how the data was distributed. Categorical variables were presented as sample numbers and percentages and compared using chi square test or the Fisher's exact test as appropriate for the distribution of the data. Two-sided p-value of <0.05 considered statistically significant. A post-hoc power analysis with the present sample size and the obtained difference in serum 25OHD levels between cases and controls yields a power of 100% to detect this difference. In fact, a difference of 2.1 ng/ml between groups already yields a potency of 89.8%. Nevertheless, due to the sample size and the lower number of events (especially mortality) in COVID-19 patients with and without vitamin D deficiency, the post-hoc power analysis for the severity endpoints was lower than 40%.
Study limitations (authors)	Problems inherent to an observational study that does not permit to establish whether vitamin D is simply a biomarker of exposure or a biomarker of effect on the disease. Other vitamin D-related parameters such as the free fraction of 25OHD, 1,25 dihydroxyvitamin D, and vitamin D-binding protein were not measured. The number of COVID-19 patients who were on oral vitamin D supplements is too small and on different dosages to draw solid conclusions on its role in the clinical outcomes of the disease. The study was conducted in a single Spanish tertiary-care hospital, and data may not be generalized to other settings, ethnicities, or countries, especially those with specific policies for vitamin D supplementation or food fortification. The methods to assess serum 25OHD levels in cases and controls were different, although there was a very good correlation between both techniques. No dietary assessment has been carried out, and therefore information on dietary habits is lacking.

	There could be differences in the clinical decisions made before hospitalisation and ICU admission due to this study not being in the UK and changes over the course of the pandemic.

Study arms

COVID-19 positive (N = 197) Patients aged 18 or over with confirmed COVID-19 admitted to hospital who are not taking vitamin D supplements.
COVID-19 positive on vitamin D (N = 19) COVID-19 positive patients who were taking vitamin D supplements for at least 3 months before they were admitted to hospital.
Control (N = 197) Controls who were not COVID-19 positive

Characteristics

Arm-level characteristics

	COVID-19 positive (N = 197)	COVID-19 positive on vitamin D (N = 19)	Control (N = 197)
Age			
MedianIQR	61 (47.5 to 70)	60 (59 to 75)	61 (56 to 66)
Gender Male			
Sample Size	n = 123 ; % = 63.4	n = 7 ; % = 36.8	n = 123 ; % = 62.4
Ethnicity			
Custom value	NA	NA	NA
Comorbidities			
Cardiovascular disease			
Sample Size	n = 21 ; % = 10.7	n = 3 ; % = 15.8	n = 22 ; % = 11.2
Hypertension			

	COVID-19 positive (N = 197)	COVID-19 positive on vitamin D (N = 19)	Control (N = 197)
Sample Size	n = 76 ; % = 38.6	n = 12 ; % = 63.2	n = 87 ; % = 44.2
Diabetes			
Sample Size	n = 34 ; % = 17.3	n = 0 ; % = 0	n = 31 ; % = 15.7
COPD			
Sample Size	n = 15 ; % = 7.6	n = 2 ; % = 10.5	n = 9 ; % = 4.6
Active cancer			
Sample Size	n = 7 ; % = 3.6	n = 0 ; % = 0	n = 8 ; % = 4.1
BMI			
Mean/SD	29.2 (4.7)	30.9 (6.3)	28.9 (4)
Use of immune suppressing treatments			
Sample Size	n = 16 ; % = 8.1	n = 6 ; % = 31.6	n = 2 ; % = 1
Socioeconomic status			
Custom value	NA	NA	NA
Previous history of COVID-19			
Custom value	NA	NA	NA
Other supplement use ACE1/ARA2 agents			
Sample Size	n = 58 ; % = 29.4	n = 7 ; % = 36.8	n = 47 ; % = 23.9
Timing of vitamin D measurements			
Shielding status			
Custom value	NA	NA	NA
Living in care homes			
Custom value	NA	NA	NA
Vitamin D *(ng/mL)*			
Mean/SD	13.8 (7.2)	21.1 (5.9)	20.9 (7.4)
Current smoker			
Sample Size	n = 14 ; % = 7.1	n = 2 ; % = 10.5	n = 34 ; % = 17.3

Outcomes

Main characteristics of COVID-19 patients according to the presence of vitamin D deficiency.

Includes comparisons between vitamin D deficient and sufficient participants in the COVID-19 not taking vitamin D supplementation only.

	Deficient 25(OH)D <20 ng/ml;	Sufficient 25(OH)D ≥20 ng/ml	p value
	N = 162	N = 35	
Age *Polarity: Not set*			
MedianIQR	62 (48 to 70.3)	58.4 (45 to 69)	0.29
Sex Male *Polarity: Not set*			
Sample Size	n = 106 ; % = 65.4	n = 17 ; % = 48.6	0.062
BMI *(kg/m²)* *Polarity: Not set*			
Mean/SD	29 (4.9)	29.8 (4.1)	0.43
Comorbidities *Polarity: Not set*			
Cardiovascular disease			
Sample Size	n = 21 ; % = 13	n = 0 ; % = 0	0.029
Hypertension			
Sample Size	n = 68 ; % = 42	n = 8 ; % = 22.9	0.035
COPD			
Sample Size	n = 13 ; % = 8	n = 2 ; % = 5.7	0.99
Active cancer			
Sample Size	n = 7 ; % = 4.3	n = 0 ; % = 0	0.36
Immunosuppression			
Sample Size	n = 11 ; % = 6.8	n = 5 ; % = 14.3	0.17
Diabetes			
Sample Size	n = 28 ; % = 17.3	n = 6 ; % = 17.1	0.98

	Deficient 25(OH)D <20 ng/ml;	Sufficient 25(OH)D ≥20 ng/ml	p value
	N = 162	N = 35	
Pneumonia *Polarity: Not set*			
Sample Size	n = 155 ; % = 95.7	n = 33 ; % = 94.3	0.66
PaO2/FIO2 ratio *(PaFI)* *Polarity: Not set*			
MedianIQR	444 (424 to 452)	444 (436 to 452)	0.17
ACE1/ARA2 agents *Polarity: Not set*			
Sample Size	n = 52 ; % = 32.1	n = 6 ; % = 17.1	0.078
Tocilizumab *Polarity: Not set*			
Sample Size	n = 55 ; % = 34	n = 8 ; % = 22.9	0.2
ICU admission *Polarity: Not set*			
Sample Size	n = 44 ; % = 27.2	n = 6 ; % = 17.1	0.22
Mechanical ventilation *Polarity: Not set*			
Sample Size	n = 37 ; % = 84.1	n = 6 ; % = 100	0.58
Secondary infection *Polarity: Not set*			
Sample Size	n = 38 ; % = 23.5	n = 6 ; % = 17.1	0.42
Death *Polarity: Not set*			
Sample Size	n = 16 ; % = 10.2	n = 4 ; % = 11.4	0.77
Composite severity endpoint *Polarity: Not set*			
Sample Size	n = 111 ; % = 68.5	n = 27 ; % = 77.1	0.31
Length of stay *Polarity: Not set*			
MedianIQR	12 (8 to 17)	8 (6 to 14)	0.013
25(OH)D			

	Deficient 25(OH)D <20 ng/ml;	Sufficient 25(OH)D ≥20 ng/ml	p value
	N = 162	N = 35	
Polarity: Not set			
Mean/SD	13.8 (7.2)	21.1 (5.9)	

Main features in COVID-19 patients with or without oral vitamin D supplements at admission.

	COVID-19 positive	COVID-19 positive on vitamin D	Control
	N = 197	N = 19	
Pneumonia *Polarity: Not set*			
Sample Size	n = 188 ; % = 95.4	n = 18 ; % = 94.7	0.99
PaO2/FIO2 ratio *Polarity: Not set*			
MedianIQR	44 (428 to 452)	444 (432 to 452)	0.52
PaFi <300 *Polarity: Not set*			
Sample Size	n = 52 ; % = 26.4	n = 1 ; % = 5.3	0.049
Tocilizumab *Polarity: Not set*			
Sample Size	n = 63 ; % = 32	n = 1 ; % = 5.3	0.015
ICU admission *Polarity: Not set*			
Sample Size	n = 50 ; % = 25.4	n = 1 ; % = 5.3	0.05
Mechanical ventilation *Polarity: Not set*			
Sample Size	n = 43 ; % = 86	n = 1 ; % = 5.3	1
Secondary infection *Polarity: Not set*			
Sample Size	n = 44 ; % = 22.3	n = 2 ; % = 10.5	0.38
Death *Polarity: Not set*			
Sample Size	n = 20 ; % = 10.4	n = 2 ; % = 10.5	1

	COVID-19 positive	COVID-19 positive on vitamin D	Control
	N = 197	N = 19	
Composite severity endpoint *Polarity: Not set*			
Sample Size	n = 59 ; % = 29.9	n = 3 ; % = 15.8	0.19
Length of stay (days) *Polarity: Not set*			
MedianIQR	12 (8 to 16)	8 (6 to 14)	0.11

Relationship between serum vitamin D levels and composite severity endpoint

Within the COVID-19 positive arm who were not supplemented with vitamin D, vitamin D levels are correlated with disease severity. The OR corresponds to a unit of vitamin D in 1 ng/ml. Adjusted model used age, smoking, hypertension, diabetes mellitus, history of cardiovascular events, immunosuppression, body mass index, serum corrected calcium, glomerular filtration rate and the month of vitamin D determination.

	COVID-19 positive vs COVID-19 positive
	N1 = 197, N2 = 197
Composite severity endpoint *Polarity: Lower values are better*	
Unadjusted	
Odds ratio/95% CI	1.55 (0.66 to 3.65)
Adjusted	
Odds ratio/95% CI	1.13 (0.27 to 4.77)

Vitamin D levels in COVID-19 patients and controls.

Multivariable general linear model adjusted for age, smoking, hypertension, diabetes mellitus, history of cardiovascular events, immunosuppression, body mass index, serum corrected calcium, glomerular filtration rate and the month of vitamin D determination. Only includes COVID-19 patients not on vitamin D supplementation, and controls.

	COVID-19 positive	COVID-19 positive on vitamin D	Control
	N = 197	N = 19	
Vitamin D level *(ng/mL)* *Polarity: Not set*			

	COVID-19 positive	COVID-19 positive on vitamin D	Control
	N = 197	N = 19	
Mean/95% CI	11.9 (9.6 to 14.3)	21.2 (19.7 to 22.7)	0.01

Section	Question	Answer
Study participation	Summary Study participation	Moderate risk of bias *(Controls only sex-matched with cases and did not control or report ethnicity, which has been shown to be associated with vitamin D status and COVID-19 morbidity and mortality.)*
Study Attrition	Study Attrition Summary	Low risk of bias *(No attrition reported.)*
Prognostic factor measurement	Prognostic factor Measurement Summary	High risk of bias *(Two different assays with using different methods were used to measure serum 25(OH)D for cases and controls. Different assays have different limits of detection and chemical concentrations cannot be compared directly to one another unless the data has been transformed in a certain way, e.g. diagnostic test accuracy metrics.)* Low risk of bias *(Between vitamin D supplemented and non-vitamin D supplemented cases, the method is the same, so analyses comparing these should be considered at low risk of bias for this domain.)*
Outcome Measurement	Outcome Measurement Summary	Low risk of bias *(HCP knowing the vitamin D status of participants in hospital unlikely to affect how they assessed patients as they were unaware the data would be used for this study at the time of assessment.)*
Study Confounding	Study Confounding Summary	Moderate risk of bias *(Unsure if the study encountered missing data or how they accounted for it. Non-reporting of confounders/baseline characteristics has been covered in another domain so has not contributed to the overall decision of this domain.)*
Statistical Analysis and Reporting	Statistical Analysis and Presentation Summary	Low risk of bias
Overall risk of bias and directness	Risk of Bias	High *(High risk of bias for data comparing cases and controls due to different methods of measuring vitamin D.* *Note: moderate risk of bias for data that compares the vitamin D supplemented and non-supplemented arms because vitamin D was measured in the same way.)*

Section	Question	Answer
	Directness	Directly applicable *(Note: there could be differences in the clinical decisions made before hospitalisation and ICU admission due to this study not being in the UK and changes over the course of the pandemic)*