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Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalised with COVID-19 are associated with greater disease severity

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Short title: Vitamin D deficiency in COVID-19 inpatients

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SUMMARY

Objectives: Vitamin D deficiency (VDD) has been proposed to play a role in Coronavirus Disease 2019 (COVID-19) pathophysiology. We aim to evaluate our implementation of a local protocol for treatment of VDD among patients hospitalized for COVID-19; to assess the prevalence of VDD among COVID-19 inpatients, and examine potential associations with disease severity and fatality.

Design and Participants: We conducted a retrospective interim audit of a local clinical care pathway for 134 inpatients with COVID-19. Prevalence of VDD, compliance with local treatment protocol and relationship of baseline serum 25(OH)D with markers of COVID-19 severity and fatality were analysed.

Results: 55.8% of eligible patients received Colecalciferol replacement, albeit not all according to the suggested protocol. Patients admitted to ITU were younger than those managed on medical wards (61.1 years ± 11.8 *vs.* 76.4 years ± 14.9, respectively, p<0.001), with greater prevalence of hypertension, higher baseline respiratory rate, National Early Warning Score-2 and C-Reactive protein level. While mean serum 25(OH)D levels were comparable (p=0.3), only 19% of ITU patients had 25(OH)D levels greater than 50 nmol/L *vs.* 39.1% of non-ITU patients (p=0.02). However, there was no association with fatality, potentially due to small sample size and prompt diagnosis and treatment of VDD.

Conclusions: Higher prevalence of VDD was observed in patients requiring ITU admission compared to patients managed on medical wards. Larger prospective studies and/or clinical trials are needed to validate and extend our observations.

Keywords: Coronavirus, Covid-19, acute respiratory distress syndrome, vitamin D, colecalciferol

LETTER

Dear Editor,

The global pandemic of Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is associated with higher fatality in respect of male sex, ageing, obesity, diabetes, hypertension, climatic factors (low ambient temperature and high geographic latitude) and, in the UK and North America, with darker-skinned ethnicities [1]; in all of which circumstances vitamin D deficiency (VDD) is more common [2,3].

Vitamin D3 is a pre-pro-hormone, whose biosynthetic pathway begins with solar UVB irradiation of 7-dehydrocholesterol in bare skin exposed to strong sunlight, and exhibits multifaceted effects beyond calcium and bone metabolism. Vitamin D receptors are highly-expressed in B- and T-lymphocytes, suggesting a role in modulating innate and adaptive immune responses [4]. 25(OH)D levels reach their nadir at the end of winter and low levels are associated with increased risk of acute respiratory tract infections during winter [5]; mitigated by vitamin D supplementation. Clinical trials involving vitamin D supplementation in COVID-19 are ongoing, but may not report within the time-frame of this pandemic.

As NorthEast England has a high prevalence of seasonal VDD [6], physicians in Newcastle-upon-Tyne Hospitals (NuTH) decided to measure admission serum 25(OH)D levels in patients with COVID-19, so to inform a treatment protocol adjusted according to the severity of baseline deficiency and based on pharmacokinetic data from Romagnoli, *et al.* [7] (Appendix 1). We audited this protocol as soon as practicable (Clinical Governance & Audit Registration N$^{o.}$10075), to determine whether data supported its continuation and whether there might also be lessons for a wider audience.

Serum 25(OH)D levels were measured in 134 (largely Caucasian) inpatients with positive SARS-CoV-2 swab or clinic-radiological diagnosis of COVID-19. A cut-off of >50nmol/L was defined as normal. Patients with VDD were treated wherever possible. No adverse effects, such as hypercalcaemia, were reported after treatment. Clinical observations at presentation (NEWS-2 score, heart rate, respiratory rate, blood pressure and temperature), and markers of inflammatory response [C-reactive protein (CRP), procalcitonin] were retrieved from electronic records. Sicker patients were admitted to Intensive Therapy Unit (ITU) and milder cases, or those with ward-based ceilings of care managed on medical wards ("non-ITU group"). Final outcome was recorded as discharge or death. Statistical methods are described in Appendix 2.

Patient characteristics are summarized in Table 1. The majority) of COVID-19 inpatients (i.e 90/134 patients or 66.4%) had vitamin D insufficiency (25-50 nmol/L); 50/134 (37.3%) were deficient (<25 nmol/L) and 29/134 (21.6%) had severe deficiency (≤15 nmol/L).

ITU patients were younger, (61.1 years ± 11.8 *vs.* non-ITU: 76.4 years ± 14.9, p<0.001), more frequently hypertensive, and had higher NEWS-2 scores (p=0.01), respiratory rate and CRP levels at presentation (Table 1). 25(OH)D levels were not associated with increased oxygen requirements, NEWS-2 score, COVID-19 radiological findings, CRP levels, or presence of co-morbidities (p>0.05).

ITU patients had lower 25(OH)D levels compared to non-ITU patients despite being younger, (33.5 nmol/L ± 16.8 *vs.* non-ITU: 48.1 nmol/L ± 38.2; mean difference for logarithmically-transformed-25(OH)D: 0.14; 95% Confidence Interval (CI): -0.15, 0.41), albeit not reaching statistical significance (p=0.3) possibly due to limited sample size. Nevertheless, ITU patients exhibited a significantly higher prevalence of VDD, with only 19% being vitamin D replete compared to 39.1% of non-ITU patients (p=0.02).

Overall, 63/113 (55.8%) of eligible patients received treatment. Of these, 33/63 patients (52.4%) were treated as per protocol and the rest given lower doses. Outcome data was available for 110/134 patients (82.1%) at the time of reporting. 94 (85.5%) patients were discharged, 16 (14.5%) died; and 24 are still receiving inpatient care. Serum 25(OH)D levels were not associated with mortality [95% CI 0.97 (0.42, 2.23), p=0.94]. Further adjustments for potential covariates including age, gender, comorbidities and CRP levels did not affect these results.

Mortality from COVID-19 is caused by severe acute respiratory syndrome, with cytokine storm and diffuse micro- and macrovascular thrombosis. Vitamin D may reduce severity of respiratory tract infections via three putative mechanisms: maintaining tight junctions, killing enveloped viruses through induction of cathelicidin and defensins, and reducing pro-inflammatory cytokine production, thereby decreasing risk of cytokine storm [8]. Therefore identifying and treating VDD may represent a promising modality for mitigating COVID-19-associated fatality.

Previous publications have highlighted potential associations between VDD and COVID-19 mortality [9]. We found no significant association between VDD and mortality, which was not unexpected given our proactive treatment protocol, small sample size and observational nature of our analysis.

In a small US study, 84.6% (11/13) ITU patients had VDD compared to 57.1% of patients on medical wards [10]. Only 19% of our ITU patients were vitamin D replete, despite being significantly younger and having fewer VDD-associated co-morbidities; challenging the dogma that VDD is a problem of the elderly. This may have implications for public health advice, especially given recent limitations on sun exposure resulting from lockdown measures.

A recent study from UK biobank found no association between serum 25(OH)D and risk of COVID-19 infection, but likewise found no association with hypertension and diabetes both well-established risk factors for fatality and, moreover, sample collection was not standardised for late winter, when the UK's COVID-19 outbreak began [11].

This is the first report exploring serum 25(OH)D levels in COVID-19 inpatients in Europe. VDD was more prevalent among patients requiring ITU admission, and thus VDD might be an under-recognized determinant of illness-severity. Strengths of our data include the acute assessment of serum 25(OH)D during COVID-19 admission. Limitations include small, non-ethnically-diverse sample and observational nature of this audit; cross-sectional analysis does not allow causality to be established, and therefore our results should be interpreted with caution.

Nevertheless, these preliminary data provide impetus to the commissioning, design and interpretation of ongoing or future clinical trials to evaluate a potential therapeutic role of vitamin D in COVID-19.

Conflict of interest: Nothing to declare.

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References

1. Khunti K, Singh AK, Pareek M, Hanif W. Is ethnicity linked to incidence or outcomes of covid-19? *Bmj.* 2020;369:m1548.
2. Hill TR, Granic A, Davies K, et al. Serum 25-hydroxyvitamin D concentration and its determinants in the very old: the Newcastle 85+ Study. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA.* 2016;27(3):1199-1208.
3. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity.* 2015;16(4):341-349.
4. Gruber-Bzura BM. Vitamin D and Influenza-Prevention or Therapy? *International journal of molecular sciences.* 2018;19(8).
5. Berry DJ, Hesketh K, Power C, Hypponen E. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *The British journal of nutrition.* 2011;106(9):1433-1440.

6. Aspell N, Laird E, Healy M, Shannon T, Lawlor B, O'Sullivan M. The Prevalence and Determinants of Vitamin D Status in Community-Dwelling Older Adults: Results from the English Longitudinal Study of Ageing (ELSA). *Nutrients.* 2019;11(6).
7. Romagnoli E, Mascia ML, Cipriani C, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *The Journal of clinical endocrinology and metabolism.* 2008;93(8):3015-3020.
8. Grant WB, Lahore H, McDonnell SL, et al. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients.* 2020;12(4).
9. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging clinical and experimental research.* 2020.
10. Lau FH, Majumder R, Torabi R, et al. Vitamin D Insufficiency is Prevalent in Severe COVID-19. *medRxiv.* 2020:2020.2004.2024.20075838.
11. Hastie CE, Mackay DF, Ho F, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. *Diabetes & metabolic syndrome.* 2020;14(4):561-565.

Appendix 1. NuTH NHS Foundation Trust treatment protocol for vitamin D deficiency in COVID-19

25(OH)D level (nmol/L)	**Dose of Colecalciferol prescribed**
Less than 13	300,000 international Units oral one-off dose Followed by 1600 international Units oral daily
13-25	200,000 international Units oral one-off dose Followed by 800 international Units oral daily
26-40	100,000 international Units oral one-off dose Followed by 800 international Units oral daily
41-74	800 international Units oral daily
Equal or greater than 75	No replacement

Appendix 2. Statistical Analyses

Statistical analysis was performed with SPSS version 26.0 (IBM Corp., Armonk, NY), as appropriate. Data are presented as mean ± standard deviation (SD), unless stated otherwise. Normality of distribution was assessed with Kolmogorov-Smirnov test and variables, including 25(OH)D and CRP levels as well as respiratory rate, WCC and lymphocyte count were logarithmically transformed for comparisons, if not normally distributed. Between group comparisons were assessed with independent t-test or Mann-Whitney U test in case of two groups, and Analysis of Variance and/or Kruskal-Wallis test in case of three or more groups. Associations between continuous variables were computed using Pearson's or Spearman's correlation coefficient and chi-square in case of categorical variables. Logistic regression models adjusting for age, gender, presence of co-morbidities and CRP levels were used to identify predictors of outcomes. Level of statistical significance was set at 0.05.

Table 1. Descriptive characteristics of audit participants.

	Non- ITU wards (N=92)	*Intensive Therapy Unit (N=42)*	*p- value*
Females (% of group subtotal)	44 (47.8%)	17 (39.5%)	0.30
Age (years)	76.4 ± 14.9	61.1 ± 11.8	**<0.001**
Ethnicity (N, %) - Caucasian - Asian - Afro-Caribbean - Other	 88 (95.7%) 3 (3.3%) 1 (1.1%) 0	 40 (95.2%) 1 (2.4%) 0 1 (2.4%)	 0.83
Comorbidities (N, %) - Hypertension - Diabetes - Obesity - Malignancy - Respiratory - Cardiovascular disease - Kidney and Liver diseases - Other	N=79 32 (40.5%) 24 (30.4%) 5 (6.3%) 12 (15.2%) 30 (38%) 15 (19%) 15 (19) 11 (13.9%)	N=35 24 (68.6%) 14 (40%) 9 (25.7%) 3 (8.6%) 12 (34.3%) 5 (14.3%) 4 (11.4%) 3 (8.6%)	 **<0.01** 0.27 **<0.01** 0.36 0.57 0.59 0.35 0.48
Systolic Blood pressure (mmHg)	125.3 ± 21.1	120.2 ± 18.5	0.18
Diastolic Blood pressure (mmHg)	71.8 ± 12.4	68.8 ± 11.5	0.22
Heart Rate (per min)	90.2 ± 20.9	92.4 ± 20.0	0.54
Respiratory Rate (per min)	21.5 ± 5.1	24.8 ± 7.0	**<0.01***
Body Temperature (^{o}C)	37.0 ± 0.9	37.5 ± 1.1	**0.02**
O_2 saturation (%)	93.1 ± 6.6	93.3 ± 4.7	0.77
White Blood Cell Count	8.9 ± 3.9	8.4 ± 3.8	0.63*
Lymphocyte Count	0.1 ± 0.6	1.3 ± 1.6	0.20*
Eosinophil	0.05 ± 0.11	0.03± 0.07	0.43
C-Reactive protein (mg/mL)	107.9 ± 92.0	143.4 ± 99.4	**0.045***
Procalcitonin (ng/mL)	0.7 ± 1.8	1.4 ± 3.1	0.90
25-hydroxyvitamin D (nmol/L)	48.1 ± 38.2	33.5 ± 16.8	0.30*
Vitamin D status (N, %)			

- <50 nmol/L	56 (60.9%)	34 (81%)	**0.02**
- ≥50 nmol/L	36 (39.1%)	8 (19%)	

*Significance is highlighted in bold. *: Ln-transformed for comparisons.*