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

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 preprohormone, 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-hydroxyvitamin D [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] and 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 North East 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 clinical/radiological diagnosis of COVID-19. A cut-off of >50 nmol/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 (ie 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, $P < .001$), more frequently hypertensive, and had higher NEWS-2 score ($P = .01$), 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 ($P > .05$ for all).

ITU patients had lower 25(OH)D levels compared with 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 ($P = .3$) possibly due to limited sample size. Nevertheless, ITU patients exhibited a significantly higher prevalence of VDD, with only 19% being vitamin D replete compared with 39.1% of non-ITU patients ($P = .02$).

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 were 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), $P = .94$]. Further adjustments for potential covariates including age, gender, co-morbidities 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

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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%)	.30
Age (years)	76.4 ± 14.9	61.1 ± 11.8	**<.001**
Ethnicity (N, %)			
Caucasian	88 (95.7%)	40 (95.2%)	.83
Asian	3(3.3%)	1 (2.4%)	
Afro-Caribbean	1 (1.1%)	0	
Other	0	1 (2.4%)	
Co-morbidities (N, %)	N = 79	N = 35	
Hypertension	32 (40.5%)	24 (68.6%)	**<.01**
Diabetes	24 (30.4%)	14 (40%)	.27
Obesity	5 (6.3%)	9 (25.7%)	**<.01**
Malignancy	12 (15.2%)	3 (8.6%)	.36
Respiratory	30 (38%)	12 (34.3%)	.57
Cardiovascular disease	15 (19%)	5 (14.3%)	.59
Kidney and liver diseases	15 (19)	4 (11.4%)	.35
Other	11 (13.9%)	3 (8.6%)	.48
Systolic blood pressure (mm Hg)	125.3 ± 21.1	120.2 ± 18.5	.18
Diastolic blood pressure (mm Hg)	71.8 ± 12.4	68.8 ± 11.5	.22
Heart rate (per min)	90.2 ± 20.9	92.4 ± 20.0	.54
Respiratory rate (per min)	21.5 ± 5.1	24.8 ± 7.0	**<.01***
Body temperature (°C)	37.0 ± 0.9	37.5 ± 1.1	**.02**
O_2 saturation (%)	93.1 ± 6.6	93.3 ± 4.7	.77
White blood cell count	8.9 ± 3.9	8.4 ± 3.8	.63*
Lymphocyte count	0.1 ± 0.6	1.3 ± 1.6	.20*
Eosinophil	0.05 ± 0.11	0.03 ± 0.07	.43
C-Reactive protein (mg/mL)	107.9 ± 92.0	143.4 ± 99.4	**.045***
Procalcitonin (ng/mL)	0.7 ± 1.8	1.4 ± 3.1	.90
25-hydroxyvitamin D (nmol/L)	48.1 ± 38.2	33.5 ± 16.8	.30*
Vitamin D status (N, %)			
<50 nmol/L	56 (60.9%)	34 (81%)	**.02**
≥50 nmol/L	36 (39.1%)	8 (19%)	

Note: Significance is highlighted in bold.

*Ln-transformed for comparisons.

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 with 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 standardized 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, nonethnically 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. *Osteoporos Int*. 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. *Obes Rev*. 2015;16(4):341-349.
4. Gruber–Bzura BM. Vitamin D and influenza—prevention or therapy? *Int J Mol Sci*. 2018;19(8):2419.
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. *Br J Nutr*. 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):1253.
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. *J Clin Endocrinol Metab*. 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):988.
9. 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*. 2020.32:1195–1198.
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 Metab Syndr*. 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
<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

25(OH)D level (nmol/L)	Dose of Colecalciferol prescribed
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.

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Comments on 'Adult height after gonadotropin-releasing hormone agonist treatment in girls with early puberty: A meta-analysis'

Dear Editor,

A recent meta-analysis performed by Park et al[1] concluded that treatment with a gonadotropin-releasing hormone analog (GnRH analog) in girls with early puberty (EP) might be effective in increasing height and that this is likely to be further influenced by the treatment duration rather than the baseline age. This conclusion is different from that noted in our systematic review, which assessed the effectiveness of puberty blockade in girls with EP but found no evidence from controlled studies that the use of GnRH analogs improved adult height.[2]

Thus, we have highlighted three aspects of this meta-analysis justifying the reasons for why this conclusion is different from ours.

First, in the title, abstract, introduction and discussion, the authors mention that they have performed their evaluations in the EP population. However, in the eligibility criteria, they state as review participants girls who attained puberty before the age of 10. As mentioned in our review, EP is defined as puberty occurring at the normal age of pubertal development but with some specific features. For instance, the appearance of breasts, Tanner stage 2 or 3, with or without pubic hair, is associated with advanced bone age, accelerated growth velocity and activated hypothalamic-pituitary-gonadal axis. Although various age intervals have been defined for puberty onset in EP, the ones most commonly used are as follows: between the ages of 8 and 10 in Europe and between the ages of 7 and 9 in the United States.[2] In their introduction, Park et al[1] defined EP as the onset of pubertal signs between the ages of 8 and 10. In this case, an eligibility criterion stating that girls who had attained puberty before the age of 10 were included means that diagnostics other than EP were also evaluated. It can be observed that out of the 14 studies included in their meta-analysis, ten studies have, as eligibility criteria, central precocious puberty (CPP), the definition of which is breast development > 2, according to Tanner, before the age of 8 (Table 1). In addition, in the 10 studies on CPP, the mean chronological age at onset of puberty was around 6 years, and for the studies that only mentioned the mean age of baseline visit or when therapy was started, this parameter was around 7 years (Table 1). In total, most patients included in this meta-analysis had idiopathic CPP (10 studies, 806 participants), and only four studies were performed on girls with EP (203 participants). Additionally, two studies on EP in which a GnRH analog was compared with no treatment were not included (152 girls in both studies combined).[3,4] It is important to emphasize that there were no differences in adult height between the treated and untreated girls in these studies on EP (studies here refers to all those studies that were included by the author and those that were not).

Second, an essential component of a systematic review assessment is the evaluation of the risk of bias of each included study. The authors used RoB 1, the tool published in version 5 of the Cochrane Handbook, for this. This tool has been previously recommended for randomized clinical trials (current recommendation is to use an updated version, RoB 2), and its domains (randomization and allocation process, blinding of participants, personnel and outcome assessment, incomplete outcome data) are not applicable for the retrospective studies that were included in this meta-analysis. Conversely, some domains specific for observational studies were not evaluated, such as confounding, selection, information and reporting bias.[5] The risk of bias assessment could be done by an investigation of how methodological limitations are associated with a GnRH analog effect in the meta-analysis.[5] For example, studies with a serious or critical risk