Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(10) 4246–4257

DOI: 10.1177/0300060518792783
journals.sagepub.com/home/imr
SAGE

Vitamin D in acutely ill patients

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Abstract

Objective: To investigate 25(OH)D_3 levels and their relationship to survival in a cohort of acutely ill patients on admission to an intensive care unit.

Methods: This study enrolled acutely ill patients at admission to an intensive care unit and a group of sex- and age-matched healthy control subjects. The 25(OH)D_3 levels were measured using an enzyme immunoassay. C-reactive protein and procalcitonin levels were also measured using immunoassays.

Results: A total of 50 acutely ill patients and 50 healthy control subjects were enrolled in the study. The mean ± SEM 25(OH)D_3 levels were significantly lower in the acutely ill patients compared with the control group (11.74 ± 0.88 ng/ml versus 24.66 ± 1.60 ng/ml, respectively). The 25(OH)D_3 levels were not related to survival. An inverse relationship was observed between 25(OH)D_3 levels and C-reactive protein levels. A weak inverse relationship was also observed between 25(OH)D_3 levels and procalcitonin levels.

Conclusions: The 25(OH)D_3 levels were decreased in acutely ill patients admitted to an intensive care unit compared with healthy control subjects. 25(OH)D_3 levels may be inversely related to C-reactive protein and procalcitonin levels.

Keywords

Vitamin D, 25(OH)D_3, intensive care unit, survival, C-reactive protein, procalcitonin

Date received: 28 October 2017; accepted: 12 July 2018

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Introduction

Vitamin D is a group of secosteroid hormones involved in calcium metabolism and musculoskeletal function.[1–3] Vitamin D enhances calcium absorption from the gastrointestinal tract and influences muscle and bone function.[4] However, recently the extraskeletal effects of vitamin D have become the focus of considerable research interest.[5,6] The relationship between vitamin D and the immune system[7–10] and metabolism[11] has been studied extensively. Vitamin D has been shown to exert multiple effects on the immune system[7–10] and to affect glucose metabolism.[11,12] Vitamin D has immune enhancing effects.[13] As well as being able to induce immune tolerance,[14–17] vitamin D deficiency has been shown to be related to the development of autoimmune diseases[18] such as type 1 diabetes mellitus,[19] multiple sclerosis[20–22] as well as rheumatoid arthritis.[23] However, vitamin D may also be a weak negative acute response factor, decreasing during an acute response.[24] Vitamin D was decreased in patients being cared for in intensive care units.[25–37] Vitamin D levels were decreased in patients with acute coronary syndromes and low vitamin D levels were found to be related to a poor outcome, including mortality.[38] Vitamin D levels have been either found[29–32,34] or not found[27,33] to be related to survival in the setting of intensive care. In a study performed in Brazil,[26] low vitamin D levels in patients being cared for in an intensive care unit were related to organ dysfunction. In a study performed in South India in patients admitted to a paediatric intensive care unit, vitamin D deficiency was related to higher severity illness, need for mechanical ventilation, more vasopressor administration and lower calcium levels.[28] In the aforementioned study,[28] vitamin D levels were not related to mortality. In a prospective cohort study in six Canadian paediatric intensive care units, low levels of 1,25 $(OH)_2D_3$ levels were observed, however no relationship with mortality was found.[30]

The aim of the current study was to investigate $25(OH)D_3$ levels and their relationship to survival in a cohort of acutely ill patients on admission to an intensive care unit.

Patients and methods

Study population

This prospective cohort study recruited consecutive acutely ill patients admitted to the intensive care unit of Alexandra Hospital, Athens, Greece between July 2016 and January 2017. Upon admission to the intensive care unit, the $25(OH)D_3$, C-reactive protein (CRP) and procalcitonin levels were measured. A control group was recruited from patients attending the hospital for various reasons who were matched for age and sex. $25(OH)D_3$ levels were also measured in the control group. Patients and controls were Caucasian from the area of central Greece. They were on a standard Greek diet; none of them reported being vegetarian or adhering to any other specific diet. None of the patients or the control group were taking vitamin D supplements. Patients and controls were recruited within a period of 6 months.

The study was approved by the Ethical Committee of St Paul's Hospital, Thessaloniki, Greece (no. 314/26-07-2016), as it was planned and initiated there. Verbal consent was taken from all the patients included in the study, either from the patient or their carer.

Biochemical analyses

A venous blood sample for the measurement of $25(OH)D_3$ was collected from each study participant at hospital admission. The blood was collected in BD

Vacutainer® red tubes (Beckton, Dickinson and Co., Franklin Lakes, NJ, USA). It was then centrifuged for 10 min at 2000 *g* in an ALC 4237 R centrifuge at 12°C (Hellenic Labware Sia, Athens, Greece). Following centrifugation, the serum was immediately transferred into a polypropylene tube (Elite Medical, Marietta, GA, USA) and stored at –20 °C until assay. Serum 25(OH)D_3 levels were measured using an enzyme immunoassay (Immunodiagnostic Systems, Tyne & Wear, UK) according to the manufacturer's instructions. In brief, 25 µl of calibrators, controls and samples were diluted with biotin-labelled 25(OH)D. The diluted samples were incubated in wells that were coated with a highly specific sheep anti-human 25(OH)D antibody at room temperature before aspiration and washing. Horseradish peroxidase-labelled avidin was added to bind selectively to complexed biotin and, following a further wash step, the colour was developed using a chromogenic substrate (3,3',5,5'-tetramethylbenzidine). The absorbance of the stopped reaction mixtures was read using a LabSystems Multiskan MS plate reader (LabSystems Diagnostics, Vantaa, Finland), colour intensity developed being inversely proportional to the concentration of 25(OH)D. The assay used was a previously validated assay.[39] The sensitivity of the assay was 1.4 ng/ml and the within-run coefficient of variance of the assay ranged from 2.3% at 45.5 ng/ml to 4.4% at 11.9 ng/ml. There was no evidence of nonlinearity.

C-reactive protein levels were measured using particle enhanced immunonephelometry according to the manufacturer's instructions (CardioPhase hsCRP; Siemens Healthcare Diagnostics, Camberley, UK). Polystyrene particles coated with monoclonal antibodies specific to human CRP were aggregated when mixed with samples containing CRP, using equipment provided by Elite Medical. These aggregates scattered a beam of light passed through the sample. The intensity of the scattered light was proportional to the concentration of the relevant protein in the sample. The result was evaluated in comparison with a standard of known concentration. The sensitivity of the assay was 0.175 mg/l with a coefficient of variation of 7.6% at 0.41 mg/l. No cross-reactivity is known for the assay. The intra-assay coefficient of variation ranged from 2.7% at 14 mg/l to 4.6% at 5.95 mg/l. The inter-assay coefficient of variation ranged from 2.0% at 14 mg/l to 4.0% at 5.95 mg/l.

Procalcitonin levels were measured using a chemiluminescent microparticle two-step immunoassay according to the manufacturer's instructions (ARCHITECT B.R.A.H.M.S. PCT; Abbott Diagnostics, Lake Forest, IL, USA). The samples were combined with paramagnetic particles covered with antibodies to procalcitonin, using equipment provided by Elite Medical. Procalcitonin within the sample was conjugated to microparticles covered with antibodies to procalcitonin. After washing, a conjugating solution of procalcitonin antibody with acridine staining was added. After a second washing cycle, photoactivation solutions from the kit were added. The chemiluminescence reaction produced was measured by relative luminescence units. The sensitivity of the assay was 0.01 µg/l. The within-run coefficient of variance ranged from 1.5% to 2.7% and the between-day coefficient of variance ranged from 2.3% to 2.8%. There was no evidence of nonlinearity. Cross-reaction with human calcitonin ranged from 0.5% to 0.7%.

Statistical analyses

All statistical analyses were performed using the IBM SPSS® statistical package, version 19.0 (IBM Corp., Armonk, NY, USA) for Windows®. Data are presented as mean ± SEM. Student's *t*-test was used to compare the patient group with the

control group. Regression analysis was performed to analyse the relationship between $25(OH)D_3$ levels, CRP and procalcitonin levels. A P-value < 0.05 was considered statistically significant.

Results

This study enrolled a cohort of 50 acutely ill patients (mean ± SD age, 66.5 ± 14.2 years; range, 24–92 years; 28 males, 22 females) who were admitted to an intensive care unit. Demographic and clinical characteristics of the patients are shown in Table 1. Amongst the cohort of acutely ill patients, four (8%) met the criteria for septic shock. Observations were also performed on a cohort of 50 age- and sex-matched healthy control subjects (mean ± SD age, 64.0 ± 14.2 years; range, 21–86 years; 26 males and 24 females).

Within the cohort of 50 patients on admission to an intensive care unit, $25(OH)D_3$ levels were significantly lower compared with the control group (mean ± SEM 11.74 ± 0.88 ng/ml, range 4.01–30.05 ng/ml, 95% confidence interval (CI) 10.09, 13.71 ng/ml and mean ± SEM 24.66 ± 1.60 ng/ml, range 7.01–55.97 ng/ml, 95% CI 21.76, 27.92 in the patient and control groups, respectively; Student's t-test, $P < 0.001$) (Figure 1).

Within the cohort of 50 patients on admission to an intensive care unit, the mean ± SEM CRP levels were 147.12 + 21.14 mg/l (range, 0.00–637.20 mg/l; normal values < 5.00 mg/l) and the mean ± SEM procalcitonin levels were 7.90 ± 2.30 ng/ml (range, 0.01–81.72 ng/ml; normal values < 0.25 ng/ml).

Levels of $25(OH)D_3$ were inversely correlated with CRP levels (correlation coefficient –0.016; 95% CI –0.015, 0.09) (Figure 2). Levels of $25(OH)D_3$ were weakly inversely correlated with procalcitonin levels (correlation coefficient –0.008; 95% CI –0.07, 0.15) (Figure 3).

There was no relationship between 25 $(OH)D_3$ levels on admission to the intensive care unit and survival, as the mean ± SEM $25(OH)D_3$ levels were 11.82 + 0.92 ng/ml and 11.50 + 2.42 ng/ml in the groups of patients who survived (mean ± SD age, 67.5 ± 12.4 years; range, 24–92 years; 22 males, 17 females) and those who did not (mean ± SD age, 62.9 ± 19.6 years; range, 24–84 years; six males, five females) respectively (odds ratio 1.008; 95% CI 0.902, 1.127) (Figure 4).

Discussion

This present study found that $25(OH)D_3$ levels were significantly decreased in a cohort of acutely ill patients on admission to an intensive care unit compared with healthy control subjects. The $25(OH)D_3$ levels were inversely related to CRP levels. There was no significant difference in $25(OH)D_3$ levels on admission to the intensive care unit between survivors and non-survivors.

Vitamin D is a group of hormones that are involved in calcium metabolism and the regulation of the musculoskeletal system.[1,2] Vitamin D affects musculoskeletal health by regulating bone and muscle health.[2] However, vitamin D also has extraskeletal effects.[5] Its effects on the immune system have been extensively investigated, with it having immune enhancing effects and it being able to induce immune tolerance.[8,9] Vitamin D deficiency has been shown to be related to the development of autoimmune diseases, such as rheumatoid arthritis[40] and systemic lupus erythematosus.[41] Vitamin D deficiency has also been found to be related to the development and severity of multiple sclerosis.[20–22] Vitamin D also affects glucose metabolism and is related to blood glucose regulation in type 2 diabetes mellitus.[11,12]

In many reports from around the world, vitamin D deficiency has been observed in

Table 1. Sex, age, diagnosis and APACHE II score on admission to the intensive care unit with the outcome (1=recovery, 2=death) of the cohort of acutely ill patients enrolled in this study ($n = 50$).

Sex	Age, years	Diagnosis	APACHE II score	Outcome
Female	61	Type 1 diabetes mellitus, pyelonephritis, septic shock	32	2
Female	75	Pulmonary abscess, respiratory insufficiency, chronic obstructive pulmonary disease	35	2
Female	83	Pulmonary infection, pulmonary insufficiency	22	1
Male	61	Pulmonary infection, chronic obstructive pulmonary disease, cardiac insufficiency, type 2 diabetes mellitus	20	1
Male	65	Endocarditis, dilated myocardiopathy, permanent heart pacer	25	1
Male	75	Coronary artery disease	21	1
Male	92	Acute myocardial infarction, cardiac insufficiency, pulmonary infection	23	1
Male	81	Heart attack, septic shock	35	2
Male	39	Renal transplantation, cytomegalovirus pneumonitis	37	2
Female	44	Miller-Fisher syndrome, pulmonary insufficiency	20	1
Female	62	Bronchitis, ventricular arrhythmia	18	1
Female	49	Multiple myeloma, autologous bone marrow transplantation, granulocytopaenia, fever	37	2
Female	81	Macroglobulinemia, pneumonia	21	1
Male	24	Type 1 diabetes mellitus, diabetic ketoacidosis	22	1
Female	55	Pulmonary insufficiency, inhalation of toxic chemicals	21	1
Male	84	Parkinson's disease, gastrointestinal infection	38	2
Male	61	Chronic obstructive pulmonary disease, pulmonary insufficiency	22	1
Male	24	Massive pulmonary embolism, pregnancy, cardiac arrest	40	2
Male	67	Guillain-Barre syndrome, swallowing inability, upper extremity paresis	27	1
Male	65	Pneumonia, pulmonary insufficiency, diabetes mellitus	28	1
Female	57	Down syndrome, pulmonary insufficiency, pneumonia	22	1
Female	86	Pulmonary infection, cardiac insufficiency, chronic obstructive pulmonary disease, pulmonary insufficiency	31	1
Male	57	Autoimmune hepatitis, liver insufficiency, cirrhosis, acute respiratory distress syndrome	38	2
Female	62	Pulmonary infection, glomerulonephritis, arterial hypertensions, chronic renal insufficiency, pulmonary insufficiency	41	2
Male	64	Cardiac insufficiency, glomerulonephritis, pulmonary infection, acute renal failure, diabetes mellitus, lactic acidosis	28	1
Female	76	Subarachnoid haemorrhage	22	1
Male	78	Multiple myeloma, cardiac insufficiency, tachyarrhythmia	38	2
Male	75	Multiple myeloma, pulmonary infection, chronic renal failure	21	1
Male	61	Acute respiratory distress syndrome, pulmonary infection	25	1
Male	82	Motor neuron disease	37	1
Male	56	Multiple myeloma, plasmatocytic leukaemia, septic shock	25	1
Male	82	Metastatic renal cancer	45	2
Female	57	Fracture of the left humerus, septicaemia	32	1
Female	68	Metastatic breast cancer	25	1
Male	56	Acute pancreatitis	22	1

(continued)

Table 1. Continued.

Sex	Age, years	Diagnosis	APACHE II score	Outcome
Male	75	Cardiac insufficiency	24	1
Female	78	Acute pancreatitis	26	1
Male	82	Multiple myeloma	22	1
Female	64	Metastatic breast cancer	25	1
Female	72	Septic shock	46	1
Female	63	Chronic renal failure, pulmonary infection	35	1
Male	58	Type 2 diabetes mellitus, cardiac insufficiency	22	1
Female	63	Cardiac insufficiency, permanent pacemaker	26	1
Male	76	Metastatic renal cancer	22	1
Male	78	Type 2 diabetes mellitus, pyelonephritis	26	1
Male	65	Pancreatic cancer, type 2 diabetes mellitus	21	1
Female	68	Metastatic breast cancer	18	1
Female	72	Cardiac insufficiency	23	1
Male	78	Chronic obstructive pulmonary disease, pulmonary insufficiency	26	1
Female	68	Bowel cancer, surgery for bowel resection	28	1

APACHE, acute physiology and chronic health evaluation.

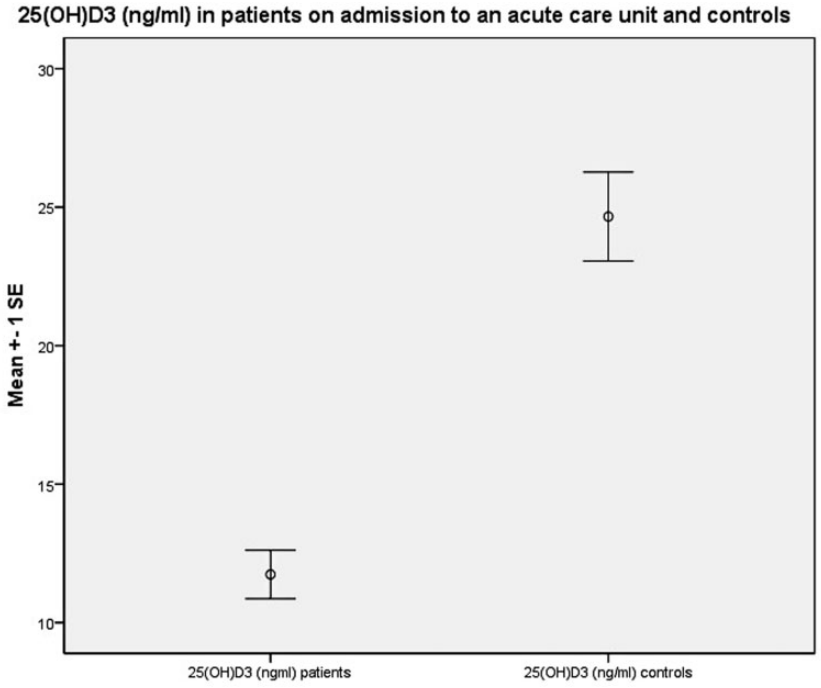

Figure 1. Levels of 25(OH)D_3 (ng/ml; mean $\pm$ SEM) in 50 acutely ill patients on admission to an intensive care unit compared with 50 age- and sex-matched healthy control subjects ($P < 0.001$; Student's *t*-test).

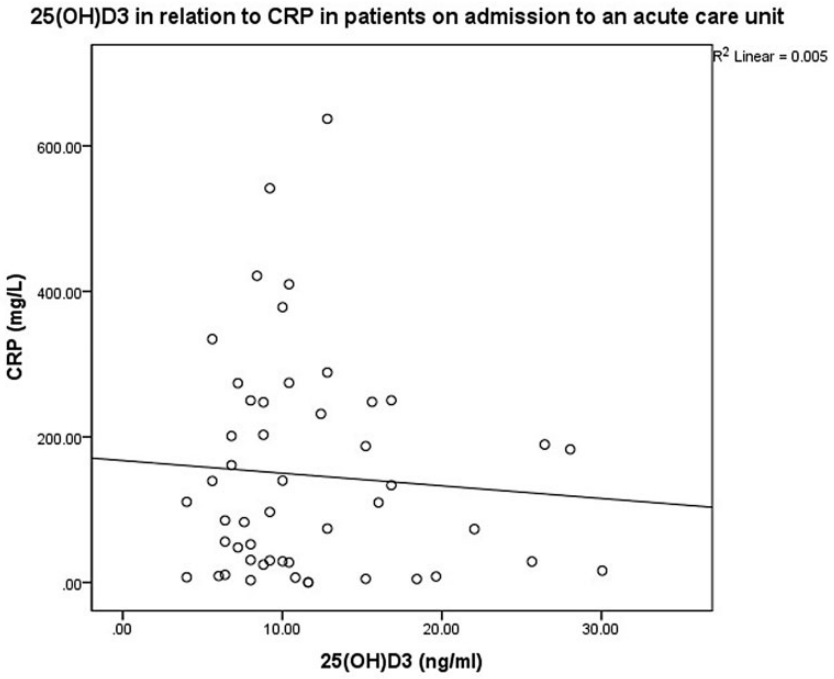

Figure 2. Scatterplot of 25(OH)D_3 in relation to C-reactive protein (CRP) in 50 acutely ill patients on admission to an intensive care unit.

patients being cared for in intensive care units.[25–37] In some of the reports, vitamin D deficiency was found to be related to survival and severe vitamin D deficiency was related to adverse outcomes after hospitalization for various reasons in intensive care units.[29–32,34] However, in other reports, vitamin D deficiency was not shown to be related to survival after hospitalization in an intensive care unit.[27,33] In a study performed in Spain, vitamin D deficiency was found to be highly prevalent in a group of 135 patients in the first 24 hours after admission to an intensive care unit.[42] The severity of the vitamin D deficiency was found to be related to adverse outcome, i.e. death and acute renal injury.[42] The cut-off point for the 25(OH)D_3 levels for the prediction of mortality was 10.9 ng/ml, which is equivalent to 27.21 nmol/l.[42] In a meta-analysis of fourteen observational studies published over a period of 14 years from 2000 to 2014 involving 9715 patients, severe vitamin D deficiency, in particular 25(OH)D_3 levels <50 nmol/l, was found to be related to severe infections, sepsis, 30-day mortality and in-hospital mortality.[32] In a study performed in France, vitamin D deficiency was found to be related to a long hospital stay in a geriatric acute care unit.[43] The same authors performed a prospective study and found that vitamin D deficiency (i.e. 25[OH]$D_3 < 25$ nmol/l) was associated with a long hospital stay in a geriatric acute care unit.[44] Thus, vitamin D deficiency was proposed as a biomarker of a long hospital stay in a geriatric acute care unit.[45] In a study performed in a neurocritical care unit on 400 patients admitted on an emergency basis, vitamin D deficiency (i.e. 25[OH]$D_3 < 20$ ng/ml; equivalent

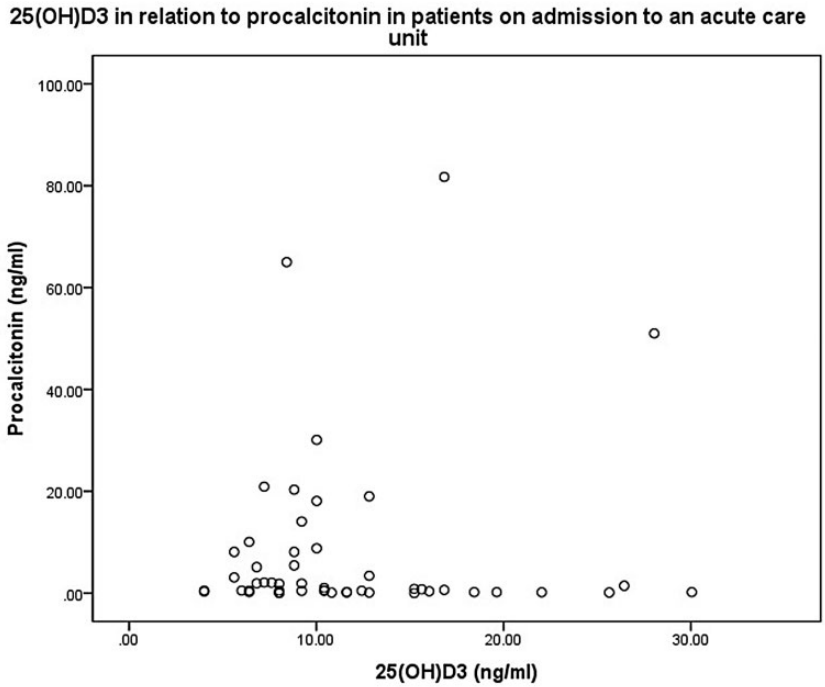

Figure 3. Scatterplot of $25(OH)D_3$ in relation to procalcitonin in 50 acutely ill patients on admission to an intensive care unit.

to < 49.92 nmol/l) was found to be associated with increased mortality.[46] It should be noted that in the present study, the mean $25(OH)D_3$ level in the acutely ill patients in intensive care was 11.74 ng/ml (equivalent to 29.3 nmol/l). Another prospective study of 497 patients admitted to a neurocritical care unit found that vitamin D deficiency was associated with worse 3-month Outcome Glasgow Scale scores.[47] A study has been designed to access the effect of vitamin D deficiency as well as vitamin D metabolism in adult acute care unit patients with and without acute kidney injury.[48] Very interestingly, and in contrast to the previous findings,[47] high levels of $1,25(OH)_2D_3$ (calcitriol) were found to be related to adverse outcome in critically ill patients with acute kidney injury.[49] In a study performed in Italy in patients admitted to an intensive care unit, low levels of $25(OH)D_3$ were observed in patients with sepsis, but no relationship with survival was observed.[50] In a study performed in China in intensive care unit patients with sepsis, low $25(OH)D_3$ levels were observed, but no relationship was found between $25(OH)D_3$ levels and survival.[51] In a study performed in the US, vitamin D deficiency was found to be related to increased length of hospital stay and mortality in surgical intensive care unit patients.[52] In a study performed in two teaching hospitals in Boston, vitamin D deficiency was found to be related to mortality and blood culture positivity in acute care unit medical and surgical patients.[53] In contrast, in a study performed in Iran, low $25(OH)D_3$ levels were observed in a large proportion (74.3%) of surgical critically ill

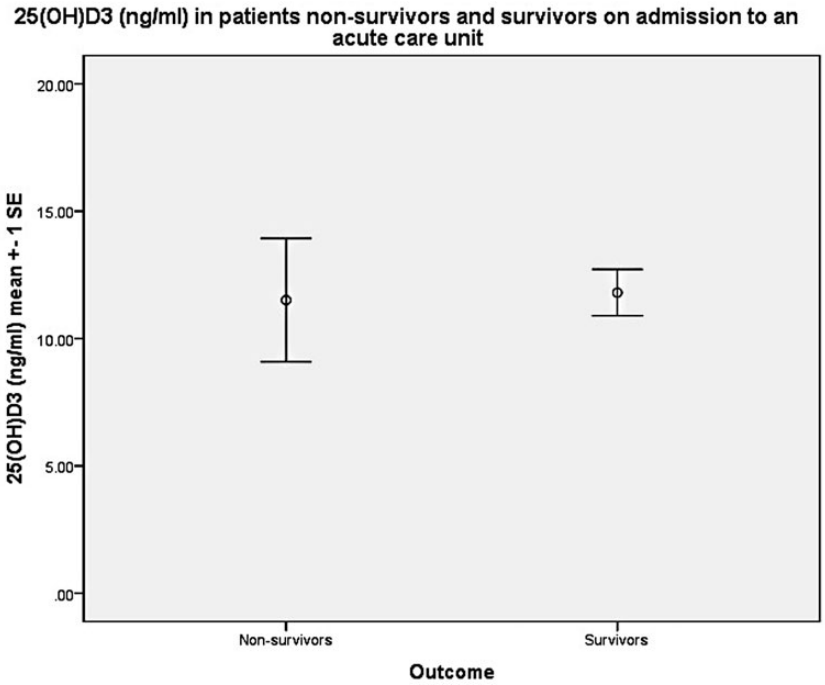

Figure 4. Levels of 25(OH)D_3 (ng/ml; mean ± SEM) in 50 acutely ill patients on admission to an intensive care unit stratified according to whether or not they survived (no significant between-group difference; Student's *t*-test).

patients.[27] Low 25(OH)D_3 levels were associated with length of hospital stay, but they were not associated with mortality.[27] In the present study, vitamin D deficiency was observed on admission to an intensive care unit compared with healthy control subjects. However, vitamin D deficiency was not related to survival, as levels of vitamin D were equally low in both survivors and non-survivors. The results of the present study demonstrate that vitamin D deficiency in the setting of the intensive care units exists and may affect immune regulation, as it has been shown to affect immunity.[7–10]

On the basis of the aforementioned studies and findings, vitamin D has been administered to critically ill patients being hospitalized in an intensive care unit to test its effects on patient hospital stay and short- and long-term mortality.[54] The administration of vitamin D_3 at high doses did not affect the length of hospital stay, hospital mortality or 6-month mortality.[54] In accordance with these findings,[54] the administration of a single intramuscular dose of cholecalciferol in intensive care unit patients did not affect in-hospital mortality.[55]

Vitamin D (25[OH]D_3) may be a negative acute phase reactant, which means that it is decreased during the acute phase response. In a study involving septic patients, 25(OH)D_3 levels were found to be decreased.[33] An inverse relationship was observed between 25(OH)D_3 and procalcitonin levels.[33] In another study performed in the UK,[56] 25(OH)D_3 levels were measured in patients who had undergone elective orthopaedic surgery and thereafter developed a systemic inflammatory

response. The $25(OH)D_3$ levels decreased after surgery as the patients developed a systemic inflammatory response.[56] In a cross-sectional population-based study performed in Brazil, an inverse association was observed between 25(OH)D levels, interleukin-6 and tumour necrosis factor-α levels in normal weight participants.[24] As a single $25(OH)D_3$ measurement may not accurately estimate vitamin D status in critically ill patients due to fluid administration and intra-day variation of $25(OH)D_3$, tissue $1,25(OH)_2D_3$ measurements in critically ill patients have been proposed for the accurate assessment of vitamin D status in this group of patients.[57] Vitamin D deficiency may have adverse consequences in critically ill patients as it is associated with immune dysregulation,[10] impaired innate immunity, impaired barrier function,[58] impaired antibacterial activity[59] and impaired endothelial function.

In conclusion, decreased $25(OH)D_3$ levels were observed in acutely ill patients at admission to an intensive care unit in Greece. Vitamin D may be inversely related to acute response indices including CRP.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Anderson PH. Vitamin D activity and metabolism in bone. *Curr Osteoporos Rep* 2017; 15: 443–449.
2. Lips P. Vitamin D physiology. *Prog Biophys Mol Biol* 2006; 92: 4–8.
3. Carlberg C. The physiology of vitamin D – far more than calcium and bone. *Front Physiol* 2014; 5: 335.
4. Bendik I, Friedel A, Roos FF, et al. Vitamin D: a critical and essential micronutrient for human health. *Frontiers in Physiology* 2014; 5: 248.
5. Bikle DD. Extraskeletal actions of vitamin D. *Ann N Y Acad Sci* 2016; 1376: 29–52.
6. Caprio M, Infante M, Calanchini M, et al. Vitamin D: not just the bone. Evidence for beneficial pleiotropic extraskeletal effects. *Eat Weight Disord* 2017; 22: 27–41.
7. Suaini NH, Zhang Y, Vuillermin PJ, et al. Immune modulation by vitamin D and its relevance to food allergy. *Nutrients* 2015; 7: 6088–6108.
8. Trochoutsou AI, Kloukina V, Samitas K, et al. Vitamin-D in the immune system: genomic and non-genomic actions. *Mini Rev Med Chem* 2015; 15: 953–963.
9. Prietl B, Treiber G, Pieber TR, et al. Vitamin D and immune function. *Nutrients* 2013; 5: 2502–2521.
10. Hewison M. Vitamin D and immune function: an overview. *Proc Nutr Soc* 2012; 71: 50–61.
11. Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, et al. Vitamin D and glycemic control in diabetes mellitus type 2. *Ther Adv Endocrinol Metab* 2013; 4: 122–128.
12. Wu C, Qiu S, Zhu X, et al. Vitamin D supplementation and glycemic control in type 2 diabetes patients: A systematic review and meta-analysis. *Metabolism* 2017; 73: 67–76.
13. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. *Curr Opin Nephrol Hypertens* 2008; 17: 348–352.
14. Clark A and Mach N. Role of vitamin D in the hygiene hypothesis: the interplay between vitamin D, vitamin D receptors, gut microbiota, and immune response. *Front Immunol* 2016; 7: 627.
15. Chirumbolo S. The role of vitamin D towards immune tolerance in white adipose tissue (WAT). *Endocr Metab Immune Disord Drug Targets* 2015; 15: 277–287.
16. Zakariaeeabkoo R, Allen KJ, Koplin JJ, et al. Are vitamins A and D important in the development of food allergy and how are they best measured? *Clin Biochem* 2014; 47: 804–811.

17. Badenhoop K, Kahles H and Penna-Martinez M. Vitamin D, immune tolerance, and prevention of type 1 diabetes. *Curr Diab Rep* 2012; 12: 635–642.
18. Zold E, Barta Z and Bodolay E. Vitamin D deficiency and connective tissue disease. *Vitam Horm* 2011; 86: 261–286.
19. Dong JY, Zhang WG, Chen JJ, et al. Vitamin D intake and risk of type 1 diabetes: a meta-analysis of observational studies. *Nutrients* 2013; 5: 3551–3562.
20. Lucas RM, Byrne SN, Correale J, et al. Ultraviolet radiation, vitamin D and multiple sclerosis. *Neurodegener Dis Manag* 2015; 5: 413–424.
21. Sundström P and Salzer J. Vitamin D and multiple sclerosis – from epidemiology to prevention. *Acta Neurol Scand* 2015; 132: 56–61.
22. Ueda P, Rafatnia F, Bäärnhielm M, et al. Neonatal vitamin D status and risk of multiple sclerosis. *Ann Neurol* 2014; 76: 338–346.
23. Kostoglou-Athanassiou I, Athanassiou P, Lyraki A, et al. Vitamin D and rheumatoid arthritis. *Ther Adv Endocrinol Metab* 2012; 3: 181–187.
24. de Souza WN, Norde MM, Oki É, et al. Association between 25-hydroxyvitamin D and inflammatory biomarker levels in a cross-sectional population-based study, São Paulo, Brazil. *Nutr Res* 2016; 36: 1–8.
25. Nair P and Venkatesh B. Vitamin D in the ICU: anything new under the sun? *Crit Care Resusc* 2012; 14: 268–273.
26. Alves FS, Freitas FG, Bafi AT, et al. Serum concentrations of vitamin D and organ dysfunction in patients with severe sepsis and septic shock. *Rev Bras Ter Intensiva* 2015; 27: 376–382.
27. Alizadeh N, Khalili H, Mohammadi M, et al. Serum vitamin D levels at admission predict the length of intensive care unit stay but not in-hospital mortality of critically ill surgical patients. *J Res Pharm Pract* 2015; 4: 193–198.
28. Ebenezer K, Job V, Antonisamy B, et al. Serum vitamin D status and outcome among critically Ill children admitted to the pediatric intensive care unit in South India. *Indian J Pediatr* 2016; 8: 120–125.
29. Onwuneme C, Carroll A, Doherty D, et al. Inadequate vitamin D levels are associated with culture positive sepsis and poor outcomes in paediatric intensive care. *Acta Paediatr* 2015; 104: e433–e438.
30. McNally JD, Menon K, Lawson ML, et al. 1,25-Dihydroxyvitamin D levels in pediatric intensive care units: risk factors and association with clinical course. *J Clin Endocrinol Metab* 2015; 100: 2942–2945.
31. Moraes RB, Friedman G, Wawrzeniak IC, et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. *Clinics (Sao Paulo)* 2015; 70: 326–332.
32. de Haan K, Groeneveld AB, de Geus HR, et al. Vitamin D deficiency as a risk factor for infection, sepsis and mortality in the critically ill: systematic review and meta-analysis. *Crit Care* 2014; 18: 660.
33. Chen Z, Luo Z, Zhao X, et al. Association of vitamin D status of septic patients in intensive care units with altered procalcitonin levels and mortality. *J Clin Endocrinol Metab* 2015; 100: 516–523.
34. Rech MA, Hunsaker T and Rodriguez J. Deficiency in 25-hydroxyvitamin D and 30-day mortality in patients with severe sepsis and septic shock. *Am J Crit Care* 2014; 23: e72–e79.
35. Zajic P and Amrein K. Vitamin D deficiency in the ICU: a systematic review. *Minerva Endocrinol* 2014; 39: 275–287.
36. Rey C, Sánchez-Arango D, López-Herce J, et al. Vitamin D deficiency at pediatric intensive care admission. *J Pediatr (Rio J)* 2014; 90: 135–142.
37. Matthews LR, Ahmed Y, Wilson KL, et al. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. *Am J Surg* 2012; 204: 37–43.
38. De Metrio M, Milazzo V, Rubino M, et al. Vitamin D plasma levels and in-hospital and 1-year outcomes in acute coronary syndromes: a prospective study. *Medicine (Baltimore)* 2015; 94: e857.
39. Carter GD, Berry JL, Gunter E, et al. Proficiency testing of 25-hydroxyvitamin D

(25OH-D) assays. *J Steroid Biochem Mol Biol* 2010; 121: 176–179.
40. Song GG, Bae SC and Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. *Clin Rheumatol* 2012; 31: 1733–1739.
41. Agmon-Levin N, Theodor E, Segal RM, et al. Vitamin D in systemic and organ-specific autoimmune diseases. *Clin Rev Allergy Immunol* 2013; 45: 256–266.
42. Zapatero A, Dot I, Diaz Y, et al. Severe vitamin D deficiency upon admission in critically ill patients is related to acute kidney injury and a poor prognosis. *Med Intensiva* 2018; 42: 216–224.
43. Beauchet O, Launay CP, Maunoury F, et al. Association between vitamin D deficiency and long hospital stay in geriatric acute care unit: results from a pilot cohort study. *Aging Clin Exp Res* 2013; 25: 107–109.
44. Beauchet O, Launay C, de Decker L, et al. Who is at risk of long hospital stay among patients admitted to geriatric acute care unit? Results from a prospective cohort study. *J Nutr Health Aging* 2013; 17: 695–699.
45. Hélard L, Mateus-Hamdan L, Beauchet O, et al. Hypovitaminosis D in geriatric acute care unit: a biomarker of longer length of stay. *Dis Markers* 2013; 35: 525–529.
46. Guan J, Karsy M, Brock AA, et al. A prospective analysis of hypovitaminosis D and mortality in 400 patients in the neurocritical care setting. *J Neurosurg* 2017; 11: 1–7.
47. Guan J, Karsy M, Brock AA, et al. Vitamin D status and 3-month Glasgow outcome scale scores in patients in neurocritical care: prospective analysis of 497 patients. *J Neurosurg* 2018; 128: 1635–1641.
48. Cameron LK, Lei K, Smith S, et al. Vitamin D levels in critically ill patients with acute kidney injury: a protocol for a prospective cohort study (VID-AKI). *BMJ Open* 2017; 7: e016486.
49. Vijayan A, Li T, Dusso A, et al. Relationship of 1,25 dihydroxy Vitamin D levels to clinical outcomes in critically ill patients with acute kidney injury. *J Nephrol Ther* 2015; 5. pii: 190.
50. Cecchi A, Bonizzoli M, Douar S, et al. Vitamin D deficiency in septic patients at ICU admission is not a mortality predictor. *Minerva Anestesiol* 2011; 77: 1184–1189.
51. Su LX, Jiang ZX, Cao LC, et al. Significance of low serum vitamin D for infection risk, disease severity and mortality in critically ill patients. *Chin Med J (Engl)* 2013; 126: 2725–2730.
52. Matthews LR, Ahmed Y, Wilson KL, et al. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. *Am J Surg* 2012; 204: 37–43.
53. Braun A, Chang D, Mahadevappa K, et al. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. *Crit Care Med* 2011; 39: 671–677.
54. Amrein K, Schnedl C, Holl A, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. *JAMA* 2014; 312: 1520–1530.
55. Nair P, Venkatesh B, Lee P, et al. A Randomized study of a single dose of intramuscular cholecalciferol in critically Ill adults. *Crit Care Med* 2015; 43: 2313–2320.
56. Waldron JL, Ashby HL, Cornes MP, et al. Vitamin D: a negative acute phase reactant. *J Clin Pathol* 2013; 66: 620–622.
57. Krishnan A and Venkatesh B. Vitamin D measurement in the intensive care unit: methodology, clinical relevance and interpretation of a random value. *Inflamm Allergy Drug Targets* 2013; 12: 230–238.
58. Hewison M, Zehnder D, Chakraverty R, et al. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. *Mol Cell Endocrinol* 2004; 215: 31–38.
59. Jeng L, Yamshchikov AV, Judd SE, et al. Alterations in vitamin D status and antimicrobial peptide levels in patients in the intensive care unit with sepsis. *J Transl Med* 2009; 7: 28.