Vitamin D in acutely ill patients

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Abstract

Objective: To investigate 25(OH)D₃ 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)D₃ 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)D₃ 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)D₃ levels were not related to survival. An inverse relationship was observed between 25(OH)D₃ levels and C-reactive protein levels. A weak inverse relationship was also observed between 25(OH)D₃ levels and procalcitonin levels.

Conclusions: The 25(OH)D₃ levels were decreased in acutely ill patients admitted to an intensive care unit compared with healthy control subjects. 25(OH)D₃ levels may be inversely related to C-reactive protein and procalcitonin levels.

Keywords
Vitamin D, 25(OH)D₃, intensive care unit, survival, C-reactive protein, procalcitonin

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**Introduction**

Vitamin D is a group of secosteroid hormones involved in calcium metabolism and musculoskeletal function. Vitamin D enhances calcium absorption from the gastrointestinal tract and influences muscle and bone function. However, recently, the extraskeletal effects of vitamin D have become the focus of considerable research interest. The relationship between vitamin D and the immune system and metabolism has been studied extensively. Vitamin D has been shown to exert multiple effects on the immune system and to affect glucose metabolism. As well as being able to induce immune tolerance, vitamin D deficiency has been shown to be related to the development of autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis as well as rheumatoid arthritis. However, vitamin D may also be a weak negative acute response factor, decreasing during an acute response. Vitamin D was decreased in patients being cared for in intensive care units. 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. Vitamin D levels have been either found or not found to be related to survival in the setting of intensive care. In a study performed in Brazil, 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. In the aforementioned study, 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)₂D₃ levels were observed, however no relationship with mortality was found.

The aim of the current study was to investigate 25(OH)D₃ 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)D₃, 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)D₃ levels were 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)D₃ 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)D₃ 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 myocardiapathy, 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, granulocytopenia, 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, tachyarrhythm                   | 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, plasmacytotic leukaemia, septic shock                  | 25              | 1       |
| Male    | 82         | Metastatic renal cancer                                                  | 45              | 2       |
| Female  | 57         | Fracture of the left humerus, septicemia                                  | 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.

![25(OH)D3 in patients on admission to an acute care unit and controls](image)

Figure 1. Levels of 25(OH)D3 (ng/ml; mean ± 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).
patients being cared for in intensive care units. 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. However, in other reports, vitamin D deficiency was not shown to be related to survival after hospitalization in an intensive care unit. 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. The severity of the vitamin D deficiency was found to be related to adverse outcome, i.e. death and acute renal injury. 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. 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. 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. 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. Thus, vitamin D deficiency was proposed as a biomarker of a long hospital stay in a geriatric acute care unit. 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 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.
to < 49.92 nmol/l) was found to be associated with increased mortality. It should be noted that in the present study, the mean 25(OH)D3 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. A study has been designed to assess the effect of vitamin D deficiency as well as vitamin D metabolism in adult acute care unit patients with and without acute kidney injury. Very interestingly, and in contrast to the previous findings, high levels of 1,25(OH)2D3 (calcitriol) were found to be related to adverse outcome in critically ill patients with acute kidney injury. In a study performed in Italy in patients admitted to an intensive care unit, low levels of 25(OH)D3 were observed in patients with sepsis, but no relationship with survival was observed. In a study performed in China in intensive care unit patients with sepsis, low 25(OH)D3 levels were observed, but no relationship was found between 25(OH)D3 levels and survival. 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. 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 adult acute care unit medical and surgical patients. In contrast, in a study performed in Iran, low 25(OH)D3 levels were observed in a large proportion (74.3%) of surgical critically ill

Figure 3. Scatterplot of 25(OH)D3 in relation to procalcitonin in 50 acutely ill patients on admission to an intensive care unit.
patients. Low 25(OH)D3 levels were associated with length of hospital stay, but they were not associated with mortality. 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.

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. The administration of vitamin D3 at high doses did not affect the length of hospital stay, hospital mortality or 6-month mortality. In accordance with these findings, the administration of a single intramuscular dose of cholecalciferol in intensive care unit patients did not affect in-hospital mortality.

Vitamin D (25(OH)D3) 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)D3 levels were found to be decreased. An inverse relationship was observed between 25(OH)D3 and procalcitonin levels. In another study performed in the UK, 25(OH)D3 levels were measured in patients who had undergone elective orthopaedic surgery and thereafter developed a systemic inflammatory

Figure 4. Levels of 25(OH)D3 (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).
response. The 25(OH)D₃ 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)D₃ measurement may not accurately estimate vitamin D status in critically ill patients due to fluid administration and intra-day variation of 25(OH)D₃, tissue 1,25(OH)₂D₃ 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 activity⁵⁹ and impaired endothelial function.

In conclusion, decreased 25(OH)D₃ 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.

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