Vitamin D status and supplementation in adult patients receiving extracorporeal membrane oxygenation

P. Nair*, B. Venkatesh†, D. J. Hoechter‡, H. Buscher§, S. Kerr**, J. R. Center††, J. A. Myburgh‡‡

Summary

The prevalence of vitamin D deficiency in critical illness is known to be high and associated with adverse clinical outcomes. Patients receiving extracorporeal membrane oxygenation (ECMO) may be at increased risk of vitamin D deficiency due to high severity of acute illness. Challenges with drug dosing in ECMO patients are recognised due to increased volume of distribution and drug absorption to circuit components. To describe the prevalence of vitamin D deficiency in ECMO patients and the effect of intramuscular dosing of cholecalciferol on levels of vitamin D metabolites, and to compare these data with intensive care unit (ICU) patients not receiving ECMO, two prospective studies were performed sequentially: an observational study of 100 consecutive ICU patients and an interventional study assessing effects of intramuscular cholecalciferol in 50 ICU patients. The subgroup of patients who required ECMO support in each of these studies was analysed and compared to patients who did not receive ECMO. Twenty-four ECMO patients, 12 from the observational study and 12 from the interventional study (who received intramuscular cholecalciferol) were studied—21/24 (88%) ECMO patients were vitamin D deficient at baseline compared to 65/126 (52%) of non-ECMO patients (P=0.006). Of the 12 ECMO patients who received cholecalciferol, six patients (50%) achieved correction of deficiency compared to 36/38 (95%) non-ECMO patients (P=0.001). The prevalence of vitamin D deficiency is higher in ECMO patients compared to other critically ill adults. Correction of deficiency with single dose cholecalciferol is not reliable; higher or repeated doses should be considered to correct deficiency.

Key Words: Vitamin D, cholecalciferol, extracorporeal life support, extracorporeal membrane oxygenation (ECMO), pharmacokinetics

Traditionally, vitamin D has been recognised for its key role in musculoskeletal health and calcium metabolism. In addition to facilitating calcium absorption from the intestine and suppressing parathyroid hormone (PTH) levels, it performs important functions in the bone.

The activated form of vitamin D is recognised by its receptor in osteoblasts causing an increase in the expression of receptor activator of nuclear factor kappa B ligand (RANKL). Its receptor, RANK on the pre-osteoclast, binds RANKL, which induces the pre-osteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralisation of the skeleton. Vitamin D deficiency is known to cause bone disease—rickets in children and osteomalacia or osteoporosis in adults[1].

Over the past decade, the pleiotropic role of vitamin D beyond its biological function in maintaining musculoskeletal health has been the subject of intense interest and research focus.

This has been driven by the finding that most body tissues have receptors for the active form of vitamin D, 1,25 dihydroxy-vitamin D [1,25 $(OH)_2D$], known as vitamin D receptors. Additionally, most of these tissues also contain the enzyme CYP27B1, which is responsible for the conversion of the major circulating form of vitamin D, 25-hydroxy-vitamin D [25(OH)D] to its active metabolite 1,25 $(OH)_2D$. Regulation of this conversion at tissue level differs from the conventional activation that occurs in the kidney in that it is more substrate dependent and hence more susceptible to vitamin D deficiency[2].

The non-skeletal, or otherwise known as pleiotropic, actions of vitamin D are mediated by the control of gene expression in a number of organs such as the brain, prostate, colon and immune cells, which may be of particular relevance in critical illness. These non-skeletal actions result in regulation of cellular proliferation, differentiation, apoptosis and

* MBBS MD FCICM, Senior Specialist, Intensive Care Unit, St Vincent's Hospital; University of New South Wales; The George Institute for Global Health; Garvan Institute of Medical Research; Sydney, New South Wales
† MBBS MD(Int.Med) FRCA FFARCSI MD(UK) FCICM, Director, Department of Intensive Care, Wesley Hospital; Pre-eminent specialist, Princess Alexandra Hospital; Professor of Intensive Care, University of Queensland; Brisbane, Queensland; Honorary Professor, University of New South Wales; Professorial Fellow, The George Institute for Global Health; Sydney, New South Wales
‡ MD, Anaesthetist, Department of Anesthesiology, Ludwig Maximilian University, Munich, Germany
§ DEAA EDIC FCICM, Senior Specialist, Department of Intensive Care, St Vincent's Hospital; Conjoint Senior Lecturer, University of New South Wales; Sydney, New South Wales
** PhD, Associate Professor, Kirby Institute, University of New South Wales, Sydney, New South Wales
†† FRACP PhD, Senior Specialist, Department of Endocrinology, St Vincent's Hospital; Professor of Endocrinology, University of New South Wales; Garvan Institute of Medical Research; Sydney, New South Wales
‡‡ MBBCh PhD FCICM FAICD FAHMS, Director, Critical Care and Trauma Division, George Institute for Global Health; Professor of Intensive Care, University of New South Wales; Senior Specialist, Intensive Care Unit, St George Private Hospital; Sydney, New South Wales
Address for correspondence: Priya Nair. Email: Priya.Nair@svha.org.au
Accepted for publication on July 11, 2018

angiogenesis[3]. In fact the mechanism of action of vitamin D in these contexts is analogous to the way in which steroid hormones act. As a result of this contemporary knowledge, vitamin D is considered more a hormone than a vitamin.

An emerging body of evidence indicates that vitamin D supplementation may be associated with reduced risk of death in diseases including multiple sclerosis, autoimmune disorders, infections, cardio-metabolic disorders and cancer[4].

Several studies have shown that the critically ill population is a vulnerable cohort with a high prevalence of vitamin D deficiency[5-10] ranging between 38% and 100%, which is 50% higher than that reported in hospitalised general medical patients[11]. Hypovitaminosis D during critical illness has been reported to be associated with adverse outcomes that include increased risk of death, longer duration of mechanical ventilation and intensive care unit (ICU) stay, increased rates of ventilator-associated pneumonia, blood culture positivity and organ dysfunction, particularly acute kidney injury[6,8,12-15].

Patients supported with extracorporeal membrane oxygenation (ECMO) fall within the extreme spectrum of illness severity in the ICU, requiring extracorporeal life support for severe respiratory and/or cardiac failure that is refractory to conventional organ support therapies. These patients spend extended periods of time in the ICU and in hospital, and frequently have underlying comorbidities prior to the index acute ICU admission. This makes them vulnerable to vitamin D deficiency when they present to the ICU, or developing this during their prolonged critical illness. Besides, ECMO therapy may change pharmacokinetics by increasing the volume of distribution (Vd), decreasing drug elimination, and sequestering drugs within the ECMO circuit. Perturbations in achieving therapeutic drug dosing of sedatives, analgesics and antibiotics have been reported[16,17].

However, there are limited data on the impact of commencement of ECMO on the Vitamin D-PTH axis.

As part of a program of vitamin D assessment and supplementation in critical illness, we undertook two serial studies of vitamin D status—an observational study of the vitamin D-PTH-calcium axis followed by a study assessing the effects of two different doses of intramuscular cholecalciferol in common clinical use. Within these two studies, patients who required ECMO support appeared to have distinct characteristics and response to supplementation. This report describes the characteristics of these ECMO patients with respect to their vitamin D status, the disposition of serum vitamin D in patients receiving ECMO, and the effects on vitamin D levels of supplementing vitamin D in comparison to critically ill patients who did not receive ECMO.

We hypothesised that:

1. There would be a greater prevalence of vitamin D deficiency in patients on ECMO as compared to the non-ECMO critically ill patient.
2. The response to a supplemental dose of vitamin D would be attenuated in the ECMO patients as compared to non-ECMO patients.

Methods

This study describes the group of patients receiving ECMO support within two previously conducted consecutive studies on vitamin D in critical illness and compares them to patients who did not receive ECMO support[8,18].

The first was a multicentre, inception observational cohort study in three tertiary centres in Sydney, New South Wales, of vitamin D status and metabolites in 100 consecutive ICU patients who were expected to stay in ICU for more than two days. Patients on renal replacement therapy were excluded.

The second study was a prospective, randomised open label interventional study of supplementation of a single dose of vitamin D (cholecalciferol), with either 150,000 IU or 300,000 IU intramuscularly, in patients who were expected to stay in the ICU for more than two days. Patients were excluded if they were pregnant, hypercalcaemic, had chronic kidney disease, had a coagulopathy (contraindicating intramuscular injection) or had a condition associated with pathological 1–alpha-hydroxylase activity.

Within these cohorts, patients who received ECMO were analysed separately. These patients were managed using a Quadrox D hollow-fibre oxygenator (Maquet® Rastatt, Germany) and a Jostra pump head (Maquet® Rastatt, Germany) for ECMO support either in a veno-venous (VV) configuration for respiratory failure, veno-arterial (VA) configuration for primary cardiac support or veno-pulmonary artery (VPA) configuration for right ventricular support following implantation of a left ventricular assist device (LVAD). Indications for commencement, management, anticoagulation, weaning and separation from ECMO were based on established protocols at a quaternary referral ECMO, VAD and cardiopulmonary transplant centre. Patients received enteral nutrition and on rare occasions, parenteral nutrition when enteral nutrition was contraindicated or could not be established successfully as per protocol. None of the patients received additional vitamin D supplementation other than that present within the nutritional solutions. (approximately 200–400 IU/day). Apart from ECMO support, patients received standard ICU care in terms of ventilatory management, vasoactive medications and antimicrobial therapies.

Data collection

In both studies, baseline demographic, diagnostic and severity of illness (Simplified Acute Physiology Score II and Acute Physiology and Chronic Health Evaluation II) data were collected on admission to the ICU.

In addition to routine blood tests performed during standard ICU care, in the observational study, serum concentrations of 25(OH)D, $1,25(OH)_2D$, intact PTH (two-

site immunoassay for intact PTH), and ionised calcium were measured at baseline and on day 3 and either at day 7 or at ICU discharge if this was prior to day 3 or 7.

In the interventional study, serum 25(OH)D and $1,25(OH)_2D$ and PTH concentrations were measured at baseline, day 1, day 3, day 7, and day 14 (or until discharge from the ICU, whichever was earlier) following administration of the cholecalciferol dose.

Assays

25(OH)D in nmol/l was measured using liquid chromatography mass spectrometry (Waters, Milford, MA, USA) in a single reference laboratory (average coefficient of variation [CV] of the three quality control [QC] levels 6.2%). $1,25(OH)_2D$ in pmol/l was measured by radioimmunoassay (DiasSorin Cat No 65100E, Saluggia, Italy) (average CV of the three QC levels 22.4%). PTH analysis was performed by immunoassay with Beckman DxI 800 immunoanalyser (Beckman Coulter, Brea, CA, USA) (average CV of the three QC levels 6.6%). Ionised calcium was measured using an ion-sensitive electrode (Radiometer ABL 720 blood gas analyser, CV 1.3%).

Vitamin D deficiency was defined as a 25(OH)D level less than 50 nmol/l (note 1 ng/ml=2.5 nmol/l). This was based on the 2010 Institute of Medicine report where a group of experts estimated that a vitamin D level of 50 nmol/l or higher was adequate for good bone health, and considered a level less than 50 nmol/l as vitamin D deficiency[19].

Statistical analysis

Continuous variables were not normally distributed and hence described as median (interquartile range, IQR). Differences between groups were tested by using Mann–Whitney U test for continuous variables or Fisher's exact test for proportions. Changes in 25(OH)D concentrations during follow-up in the 12 patients who did not receive cholecalciferol were compared with a mixed linear model.

A *P*-value of less than or equal to 0.05 was considered statistically significant.

Ethics approval

Human Research Ethics Committee (HREC) approval was obtained in the local jurisdiction separately for both studies and informed consent was obtained either from the patient or more frequently from the next of kin (Reference numbers HREC/09/SVH/165 and HREC/12/SVH/322 respectively).

Results

Twelve patients in the observational and 12 patients in the interventional study received ECMO, resulting in a total of 24 ECMO patients, 12 of whom were supplemented with cholecalciferol.

Patients in the ECMO group were younger, had a longer duration of ICU and hospital stay and a higher mortality suggesting an overall increased illness severity, although illness severity scores did not reflect this (Table 1).

Patients who required ECMO support received either VA ECMO for cardiac and respiratory support, VV ECMO for respiratory support alone, or VPA ECMO for right ventricular support following LVAD implantation (Table 2).

Table 1

Patient characteristics

	All (n=150)	ECMO patients (n=24)	Non-ECMO patients (n=126)	*P*-value
Age, years, median (IQR)	53 (40–66)	46 (33–55)	55 (41–68)	0.007*
Male gender, n (%)	101 (67%)	15 (60%)	86 (68%)	0.44
APACHE II score, median (IQR)	20 (15–25)	22 (17–30)	20 (15–25)	0.16
ICU LOS, days, median (IQR)	10 (5–17)	19 (14–29)	8 (4–14)	<0.00001*
Hospital LOS, days, median (IQR)	24 (14–40)	32 (23–57)	24 (13–34)	0.02*
Hospital mortality, n (%)	17 (11%)	8 (33%)	9 (6.5%)	0.0001*

* *P* <0.05, difference between ECMO patients and non-ECMO patients. ECMO, extracorporeal membrane oxygenation; APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; LOS, length of stay; IQR, interquartile range.

Table 2

a) Diagnostic categories for all patients at intensive care admission (n=150)

Diagnostic category	n
Infection/sepsis	52
Cardiac surgical and medical	35
Trauma	26
Neurological	7
Metabolic	7
Transplant – heart or lung	20
Miscellaneous	3

b) Indications for extracorporeal membrane oxygenation support (n=24)

Diagnostic group	n	ECMO configuration
Cardiogenic shock	10	VA
Sepsis	2	VA
Respiratory failure	4	VV
LVAD perioperative support	5	VPA
Primary graft dysfunction, heart or lung transplant	3	VA=2, VV=1

VA, veno-arterial; VV, veno-venous; VPA, veno-pulmonary artery; LVAD, left ventricular assist device; ECMO, extracorporeal membrane oxygenation.

Table 3

Vitamin D and metabolites at baseline

	All ECMO patients (n=24)	Non-ECMO patients (n=126)	*P*-value
25(OH)D, nmol/l, median (IQR)	35 (27–42)	40 (27–58)	0.02*
1,25$(OH)_2$D, pmol/l, median (IQR)	72 (46–128)	71 (47–123)	0.89
PTH, pmol/l, median (IQR)	5 (2.7–10.5)	6 (4–11)	0.9
Ionised calcium, mmol/l, median (IQR)	1.07 (1.02–1.1)	1.08 (1.02–1.14)	0.4

ECMO, extracorporeal membrane oxygenation; PTH parathyroid hormone; IQR, interquartile range.

Hormonal profiles

At baseline, 21/24 (88%) ECMO patients were vitamin D deficient. In the interventional dosing study, 5/6 (83.3%) of patients in each of the two dosing groups were deficient at baseline. Comparatively, the prevalence of deficiency in the non-ECMO patients was significantly lower at 65/126 (52%) (*P*=0.006). The median (IQR) baseline 25(OH)D level in the ECMO group was 35 (27–42) nmol/l compared with 40 (27–58) nmol/l in the non-ECMO group (*P*=0.02).

Baseline values of other parameters such as 1,25$(OH)_2$D, PTH and ionised calcium were not significantly different (Table 3).

In the 12 ECMO patients who did not receive a cholecalciferol dose, 25(OH)D levels did not change significantly from baseline to day 3 (*P*=0.37) or day 7 (*P*=0.18) (Table 4).

Of the 12 ECMO patients who received cholecalciferol supplementation, six patients (50%) achieved correction of deficiency (>50 nmol/l). In the non-ECMO patients 36 of 38 (95%) patients achieved correction of their vitamin D levels over the study duration (*P*=0.001) (Figure 1). Observed changes were similar with both doses of cholecalciferol (150,000 IU and 300,000 IU) and therefore individual dose groups are not reported separately.

The incidence of correction (defined as the achievement of a serum 25(OH)D level >50 nmol/l) in the ECMO group, as in the entire patient cohort, was no different between those receiving the 150,000 IU or 300,000 IU dose of cholecalciferol.

There were no occurrences of hypervitaminosis D,

Table 4

Serum 25(OH)D levels over time in extracorporeal membrane oxygenation patients who did not receive supplementation

Baseline Median (IQR)	Day 3 Median (IQR)	Day 7 Median (IQR)
32 (27–36)	27 (21–38)	31 (25–38)

Using linear mixed model, *P*-value for difference between at day 3 is 0.37 and at day 7 is 0.18. IQR, interquartile range.

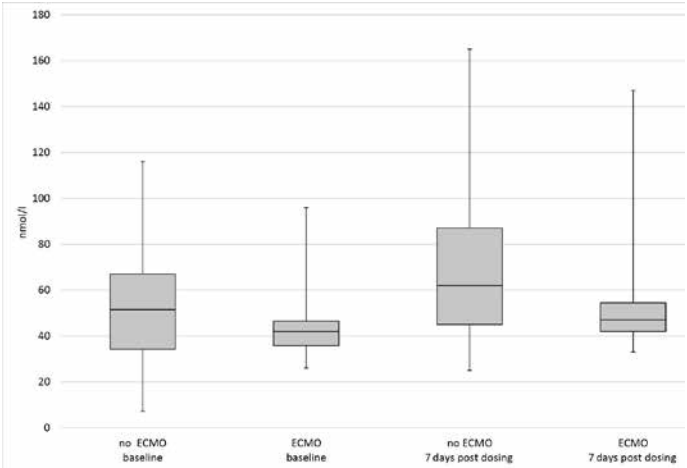

Figure 1: Serum 25(OH)D levels in the 12 ECMO patients and 38 non-ECMO patients who received cholecalciferol supplementation. ECMO, extracorporeal membrane oxygenation.

hypercalcaemia or renal calculi in the ECMO patients who received supplementation with cholecalciferol. In the non-ECMO patient group who received intramuscular cholecalciferol, one patient from the 150,000 IU group was observed to have asymptomatic hypercalcaemia (ionised calcium >1.3 mmol/l) on day 7. No other complications were seen in either group.

Discussion

This study demonstrates that severely ill patients receiving ECMO for organ support are almost universally deficient in 25(OH)D, the storage form of the vitamin used to assess vitamin D sufficiency. The concentrations of the active metabolite 1,25$(OH)_2$D were observed to be in the normal range in these patients. Administration of a single intramuscular dose of cholecalciferol resulted in normalisation of 25(OH)D concentrations in only half the study population. This is despite the fact that they were relatively younger than their counterparts who did not receive ECMO.

Vitamin D levels remained relatively static through the first week of ICU stay, suggesting that conventional nutrition provided to ICU patients does not provide adequate supplementation.

Despite this high prevalence of low 25(OH)D levels (storage form), the levels of the active metabolite 1,25$(OH)_2$D were maintained. This is because in the context of vitamin D deficiency, the consequent rise in PTH facilitates an increase in the renal conversion to 1,25 $(OH)_2$D as a compensatory mechanism. The levels of 1,25$(OH)_2$D are 1000-fold less than 25(OH)D and circulating levels are generally maintained even in the setting of severe vitamin D deficiency[20]. Furthermore, the levels of 1,25$(OH)_2$D assayed are circulating levels which do not necessarily reflect the tissue level responsible for the paracrine pleiotropic functions described previously.

The kinetics of vitamin D in critically ill patients, even without ECMO, are altered compared to a healthy population. Czarnik et al described unstable levels of vitamin D, which mirrored the severity of illness, i.e. critical instability at the beginning, steady state and then stabilisation. They postulated that the instability of the vitamin D serum concentration could also relate to a decrease in circulating albumin and vitamin D binding protein (VDBP), which typically occurs after fluid resuscitation in the ICU, decreased synthesis of VDBP, renal wasting of vitamin D, interstitial extravasation caused by increased vascular permeability following an inflammatory reaction, lack of exposure to sunlight, poor nutrition, decreased renal production of $1,25(OH)_2D$, and increased tissue conversion of 25(OH)D to $1,25(OH)_2D$[21].

A study that undertook hourly assays of vitamin D in a cohort of ICU patients demonstrated marked variability during a 24-hour period[22]. Another study using cardiopulmonary bypass as a model for haemodilution (such as might be seen in the resuscitative phase of critical illness) demonstrated that haemodilution significantly lowered serum 25(OH)D levels, which took up to 24 hours to resolve[23]. Therefore interpreting a single measurement in critically ill patients to assess deficiency risk or to consider supplementation requires caution.

ECMO support is likely to impose further perturbations on the kinetics of vitamin D.

In the group of patients who received single high dose cholecalciferol supplementation, correction of vitamin D levels was more difficult to achieve compared to those not receiving ECMO. This could be due to the altered pharmacokinetics associated with the extracorporeal circuit as well as other factors such as illness severity, intense inflammation etc.

There is a paucity of studies on pharmacokinetics in adult ECMO patients, although there have been a few studies in paediatric and neonatal populations. An ex vivo study of different neonatal and paediatric ECMO circuits described the kinetics of selected sedatives and antibiotics[24]. The investigators demonstrated that significant absorption of drugs occurs in the ECMO circuit, which correlated with increased lipophilicity of the drug. Centrifugal pump circuits with hollow-fibre membrane oxygenators show less absorption for all drugs, but the difference for lipophilic drugs is most pronounced.

A review by Shekar et al[25] described the basis of the pharmacokinetic alterations observed in ECMO patients to be due to haemodilution from priming or transfusion, sequestration or inactivation within the circuit, inflammation/sepsis and/or organ failure and drug factors such as lipophilicity. All these factors may result in alteration in the Vd, bioavailability, maximum concentration and clearance of drugs depending on their individual physicochemical properties.

Shekar et al were able to demonstrate in an ex vivo study using a standard adult ECMO circuit, that commonly used antibiotics, sedatives and analgesics were variably sequestered in the circuit with significant loss at 24 hours of fentanyl, midazolam and meropenem, but not vancomycin[16].

In another ex vivo study in ECMO patients, looking at the disposition of micro- and macronutrients, significant alterations in concentrations were described. This was particularly the case for isoleucine, an essential amino acid, and the vitamins A and E, but not D. The authors suggested that the lipophilicity of vitamins A and E might explain their loss, but were not able to explain the relatively stable levels of vitamin D[26].

Hak et al investigated PTH and calcitriol ($1,25(OH)_2D$) levels in neonatal patients receiving ECMO[27]. Hypercalcaemia was found to be a common complication in ECMO patients. With hypercalcaemia, plasma concentrations of PTH are expected to reduce with an increase in calcitonin levels. The authors observed aberrant calcitriol-endocrine regulation of calcium in these patients and postulated a number of mechanisms for the altered endocrine status. Calcitriol levels were not investigated in these patients. This and other vitamin D studies suggest that the paediatric ICU population might mirror adult critical illness including the presence of ECMO support[28,29].

Our study is an in vivo study in a critically ill cohort with severe organ failure and extracorporeal support, compared to ex vivo studies described by other authors. It is the first study to report vitamin D status and supplementation in adult ECMO patients with systematic prospective data collection, multiple measurements and precise time intervals from baseline, and describe detailed profiling of the vitamin D metabolites, PTH and calcium.

However, this study is limited with its small sample size and the heterogeneity in illness severity and indication for ECMO. Due to this small and heterogeneous group of patients, appropriate propensity matching could not be performed. As a result, a small group of patients (ECMO cohort) was compared to a larger group of critically ill patients, which does not take into consideration power calculations but provides biochemical data on a patient group with extreme pathophysiological derangements. Although vitamin D supplementation was undertaken with a single dose, we have previously shown that single-dose intramuscular cholecalciferol is a safe and effective supplementation strategy[30].

Our study showed the widespread prevalence of vitamin D deficiency in this critically ill subgroup of patients receiving ECMO. While this most likely relates to their high overall illness severity, it suggests that there may be other similar groups such as those receiving other forms of extracorporeal support who are similarly at greater risk of deficiency within a general, heterogeneous ICU population.

The difficulty in achieving therapeutic levels with

supplementation may apply to other lipophilic drugs administered to ECMO patients and therefore regular therapeutic drug monitoring is required where possible, especially for life-saving drugs. Although this study can only speculate on the mechanism of deficiency and difficulty with supplementation in this small subgroup of patients, it is hypothesis-generating and may provide a basis for further exploration into this complex area.

With the growing application of ECMO, for any future trials of vitamin D supplementation in critically ill patients, this group of patients may require to be a predefined subgroup, or excluded from such trials, to obtain meaningful results.

Conclusions

Vitamin D deficiency is even more common in ECMO patients than in a standard ICU population. Supplementation cannot be reliably achieved with conventional single-dose therapy; higher or repeated doses need to be considered to achieve target supplementation. Given the pleiotropic benefits of vitamin D, achieving adequate supplementation may be an important consideration in this group of patients with high illness severity.

Funding

The study (reference 18) from which data is included received grants for serum assays from the Intensive Care Foundation and the St Vincent's Clinic Foundation, Sydney. These funding bodies had no role in the design of the study, collection, analysis or interpretation of data, or writing of the manuscript.

Acknowledgements

The authors gratefully acknowledge the contribution of Claire Reynolds (Research Coordinator) with screening and data collection.

References

1. Wacker M, Holick MF. Vitamin D—effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 2013; 5:111-148.
2. Bikle D. Non-classic actions of vitamin D. J Clin Endocrinol Metab 2009; 94:26-34.
3. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-281.
4. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014; 21:319-329.
5. Amrein K, Zajic P, Schnedl C, Waltensdorfer A, Fruhwald S, Holl A et al. Vitamin D status and its association with season, hospital and sepsis mortality in critical illness. Crit Care 2014; 18:R47.
6. Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med 2011; 39:671-677.
7. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med 2009; 360:1912-1914.
8. Nair P, Lee P, Reynolds C, Nguyen ND, Myburgh J, Eisman JA, Center JR. Significant perturbation of vitamin D-parathyroid-calcium axis and adverse clinical outcomes in critically ill patients. Intensive Care Med 2013; 39:267-274.
9. Quraishi SA, Bittner EA, Blum L, McCarthy CM, Bhan I, Camargo CA Jr. Prospective study of vitamin D status at initiation of care in critically ill surgical patients and risk of 90-day mortality. Crit Care Med 2014; 42:1365-1371.
10. Lucidarme O, Messai E, Mazzoni T, Arcade M, du Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. Intensive Care Med 2010; 36:1609-1611.
11. Lee P. Vitamin D metabolism and deficiency in critical illness. Best Pract Res Clin Endocrinol Metab 2011; 25:769-781.
12. McKinney JD, Bailey BA, Garrett LH, Peiris P, Manning T, Peiris AN. Relationship between vitamin D status and ICU outcomes in veterans. J Am Med Dir Assoc 2011; 12:208-211.
13. Moromizato T, Litonjua AA, Braun AB, Gibbons FK, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and sepsis in the critically ill. Crit Care Med 2014; 42:97-107.
14. Braun AB, Gibbons FK, Litonjua AA, Giovannucci E, Christopher KB. Low serum 25-hydroxyvitamin D at critical care initiation is associated with increased mortality. Crit Care Med 2012; 40:63-72.
15. Braun AB, Litonjua AA, Moromizato T, Gibbons FK, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and acute kidney injury in the critically ill. Crit Care Med 2012; 40:3170-3179.
16. Shekar K, Roberts JA, Mcdonald CI, Fisquet S, Barnett AG, Mullany DV et al. Sequestration of drugs in the circuit may lead to therapeutic failure during extracorporeal membrane oxygenation. Crit Care 2012; 16:R194.
17. Shekar K, Roberts JA, McDonald CI, Ghassabian S, Anstey C, Wallis SC et al. Protein-bound drugs are prone to sequestration in the extracorporeal membrane oxygenation circuit: results from an ex vivo study. Crit Care 2015; 19:164.
18. Nair P, Venkatesh B, Lee P, Kerr S, Hoechter DJ, Dimeski G et al. A randomized study of a single dose of intramuscular cholecalciferol in critically ill adults. Crit Care Med 2015; 43:2313-2320.
19. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011; 96:53-58.
20. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19:73-78.
21. Czarnik T, Czarnik A, Gawda R, Gawor M, Piwoda M, Marszalski M et al. Vitamin D kinetics in the acute phase of critical illness: a prospective observational study. J Crit Care 2018; 43:294-299.
22. Venkatesh B, Davidson B, Robinson K, Pascoe R, Appleton C, Jones M. Do random estimations of vitamin D3 and parathyroid hormone reflect the 24-h profile in the critically ill? Intensive Care Med 2012; 38:177-179.

23. Krishnan A, Ochola J, Mundy J, Jones M, Kruger P, Duncan E, Venkatesh B. Acute fluid shifts influence the assessment of serum vitamin D status in critically ill patients. Crit Care 2010; 14:R216.
24. Wildschut ED, Ahsman MJ, Allegaert K, Mathot RAA, Tibboel D. Determinants of drug absorption in different ECMO circuits. Intensive Care Med 2010; 36:2109-2116.
25. Shekar K, Fraser JF, Smith MT, Roberts JA. Pharmacokinetic changes in patients receiving extracorporeal membrane oxygenation. J Crit Care 2012; 27:741.e9-18.
26. Estensen K, Shekar K, Robins E, McDonald C, Barnett AG, Fraser JF. Macro- and micronutrient disposition in an ex vivo model of extracorporeal membrane oxygenation. Intensive Care Med Exp 2014; 2:29.
27. Hak EB, Crill CM, Bugnitz MC, Mouser JF, Chesney RW. Increased parathyroid hormone and decreased calcitriol during neonatal extracorporeal membrane oxygenation. Intensive Care Med 2005; 31:264-270.
28. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, Doherty DR. The association of vitamin D status with pediatric critical illness. Pediatrics 2012; 130:429-436.
29. McNally JD, Nama N, O'Hearn K, Sampson M, Amrein K, Iliriani K et al. Vitamin D deficiency in critically ill children: a systematic review and meta-analysis. Crit Care 2017; 21:287.
30. Diamond TH, Ho KW, Rohl PG, Meerkin M. Annual intramuscular injection of a megadose of cholecalciferol for treatment of vitamin D deficiency: efficacy and safety data. Med J Aust 2005; 183:10-12.