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Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicentre, placebo- controlled double-blind phase III RCT (The VITDALIZE Study)

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ABSTRACT

Introduction: Observational studies have demonstrated an association between vitamin D deficiency and increased risk of morbidity and mortality in critically ill patients. Cohort studies and pilot trials have suggested promising beneficial effects of vitamin D replacement, at least in patients with severe vitamin D deficiency. As vitamin D is a simple, low-cost and safe intervention, it has potential to improve survival in critically ill patients.

Methods and analysis: In this randomised, placebo-controlled, double-blind, multicentre, international trial, 2,400 adult patients with severe vitamin D deficiency (25(OH)D ≤12ng/ml) will be randomised to receive a loading dose of 540,000 I.U. cholecalciferol within 72 hours after ICU admission, followed by 4,000 I.U. daily for 90 days or identical placebo. The primary outcome is mortality at 28 days after randomisation. Secondary outcomes are: ICU, hospital, 90 day and 1 year mortality; hospital and ICU length of stay (starting at day 0, ending at discharge from the trial site or day 90); change in organ dysfunction on day 5 as measured by Sequential Organ Function Assessment score (SOFA), number of organ failures; Hospital and ICU readmission until day 90; Discharge destination (home, rehabilitation, other hospital); self-reported infections requiring antibiotics until day 90

Ethics and dissemination: Ethical approval was obtained by the Ethics Committee of the University of Graz, and will be gained according to individual national processes. Upon completion, results will be published in a peer-reviewed scientific journal. Our broad co-applicant group will ensure rapid comprehensive dissemination. The study findings will be presented at national and international meetings with abstracts on-line. We will use Free Open Access Medical Education (FOAMed) resources to ensure as wide an audience is reached. With the help of our patient co-applicants, a lay person's summary will be sent to local and national patient support and liaison groups

Trial registration: ClinicalTrials.gov (Identifier: NCT03188796), EudraCT-No.: 2016-002460-13

STRENGTH and LIMITATIONS of the STUDY

- VITDALIZE is a large international study that will determine definitively the clinical effectiveness of vitamin D3 supplementation in critically ill patients with severe vitamin D deficiency.
- Simple method to improve patient's outcome and quality of life.
- Inclusion of a heterogeneous intensive care unit patient cohort but only treating patients with severe vitamin D deficiency and therefore most likely to benefit from treatment.

INTRODUCTION

Vitamin D has much broader effects on various metabolic activities than originally expected [1–3]. Many recent papers have demonstrated the pleiotropic effects of vitamin D. Vitamin D is a precursor of a steroid hormone with a specific nuclear receptor (vitamin D receptor), which regulates more than 1,000 genes and is also an important regulator of the immune system [4]. Besides regulating calcium homoeostasis, vitamin D has an influence on muscles, blood vessels, cell proliferation and differentiation, and autoimmune processes. Therefore, vitamin D deficiency causes skeletal and non-skeletal diseases and seems to predispose to a variety of respiratory, immune, infectious, neurologic and cardiovascular diseases. A recent Cochrane meta-analysis [5] with 50,623 adults who were healthy or were recruited among the general population, or diagnosed with a specific disease, showed that vitamin D3 supplementation was linked to significantly improved survival. An individual patient data analysis from 8 major vitamin D trials with > 70,000 participants showed a reduction of mortality by vitamin D by 9% [6]. Genetically low 25-Hydroxyvitamin D is associated with increased all-cause mortality [7]. An observational cohort study of 4,344 adults hospitalised between 1993 and 2011 demonstrated that in those patients with pre-hospital 25(OH)D concentrations <20ng/ml, an improvement in vitamin D status during the year leading up to hospitalisation was independently associated with improved all-cause mortality and decreased hospital length of stay [8].

25(OH)D is the major circulating vitamin D metabolite, and its measure best reflects an individual's vitamin D status. Thus, serum 25(OH)D is the generally accepted parameter for determining vitamin D status. Although the definition for vitamin D deficiency is still under debate, a cut-off of 25(OH)D ≤12ng/ml (=30 nmol/l) is uniformly considered to represent deficiency [9]. 25(OH)D levels below 12 ng/ml (or 30 nmol/l) hallmark a greatly increased risk for rickets, osteomalacia and decreased fractional calcium absorption. Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating of vitamin D deficiency is currently not routinely performed in the ICU.

To date, only a few studies have investigated high-dose vitamin D in critically ill patients with severe vitamin D deficiency [10–13]. The largest study to date, the VITdAL-ICU randomised, double-blind, placebo-controlled, single-centre trial included 492 critically ill patients with vitamin D deficiency (25(OH)D ≤ 20ng/ml) assigned to receive either vitamin D or placebo [10]. Vitamin D3 or placebo was given orally or via nasogastric tube at a dose of 540,000 I.U. followed by monthly maintenance doses of 90,000 I.U. for 5 months. The study provided no differences between vitamin D and placebo group concerning the primary outcome of hospital length of stay, hospital mortality or 6-month mortality. However, lower hospital mortality was observed in the severe vitamin D deficiency subgroup (25(OH)D ≤12 ng/ml, n=200 or 42% of the total population). As this was only a secondary endpoint in the

predefined subgroup with severe vitamin D deficiency, this finding is hypothesis generating and prompted the current VITDALIZE study.

METHODS and ANALYSIS

Aim

The VITDALIZE trial is a multicentre, randomised, placebo-controlled, double-blind phase III trial targeting a sample size of 2,400 critically ill patients with severe vitamin D deficiency in more than 30 sites in Austria, Germany, Belgium, Switzerland and UK.

The aim of the trial is to determine if high-dose vitamin D3 improves clinical outcomes and is cost-effective in comparison to placebo in adult critically ill patients with severe vitamin D deficiency.

Trial population

The trial population consists of mixed adult critically ill patients within 72 hours of ICU admission anticipated to require ≥ 48h of ICU care at the time of screening with documented vitamin D deficiency using local routine testing (25(OH)D ≤ 12 ng/ml (=30 nmol/l)) recruited in several countries in academic and non-academic hospitals.

A flowchart of study intervention is seen in Fig. 1 and a schedule of assessment and procedures in Table 1.

Fig.1: Trial flow of intervention scheme

We will check eligibility and obtain informed consent from patients or legally authorized representative/ health care proxy. After evaluation of exclusion criteria, patients will be randomised in the intervention or placebo group. Primary endpoint is 28-day all-cause mortality.

* When informed consent is not possible at time of screening, country-specific alternative strategies for obtaining informed consent are used (i.e. in Austria delayed informed consent, in Germany consent of relatives)

Assessment	**Screening (V0)**	**Enrolment, Baseline data (V1)**	**Clinical data (V2)**	**28-day mortality (V3)**	**90-day follow-up (V4)**	**1-year follow-up (V5)**
		Day 0	Day 5	Day 28	Day 90	Month 12
Inclusion/Exclusion criteria	X	X				
Informed consent*		X				
Demographics		X				
RANDOMISATION		X				
INTERVENTION						
Loading dose 540,000 I.U. vitamin D3		X				
Daily dose 4,000 I.U. vitamin D3			X	X	X	
OUTCOME VARIABLES						
Mortality			X	X	X	X
SOFA		X	X			
Infections requiring antibiotics					X	
Hospital and ICU readmission					X	
Katz activity of daily life		X			X	
SAFETY EVALUATION						
Serum calcium	X	X	X	(X)		
Falls/Fractures					X	
New episodes of nephrolithiasis					X	
Creatinine					X	

Table 1: Frequency and scope of study visits.

The day of the study medication loading dose is day 0 (V1). At day 5 (V2), extensive clinical data will be collected. The primary endpoint (28-day mortality) will be assessed at V3. A follow-up visit will be done by telephone 90 days after randomisation with patient or family physician. This visit will include important safety evaluations and secondary outcomes. A final follow-up visit will be done after 1 year.

Intervention

Patients will be randomised with a web-based randomisation tool (www.randomizer.at) in a 1:1 ratio using permuted blocks either to

- **Vitamin D3 group** receiving a bolus of 540,000 I.U. vitamin D3, dissolved in medium chain triglycerides (MCT, 37.5 ml), at day 0 followed by 4,000 I.U. daily (10 drops) for 90 days, or

- **Placebo group** receiving 37.5 ml MCT solution at day 0 followed by 10 drops MCT for 90 days.

A concomitant routine low dose vitamin D intake of up to 800 I.U. daily is permitted, but very unlikely to have an effect in this 90-day time frame and population.
Randomisation is stratified by centre and gender.

Inclusion and Exclusion Criteria

Inclusion criteria

- Age ≥ 18 years
- Anticipated ICU stay ≥ 48 hours
- Admission to ICU ≤ 72 hours before screening
- Severe vitamin D deficiency (≤ 12 ng/ml (30nmol/L) or undetectable) using local routine testing after ICU admission

Exclusion criteria

- Severe gastrointestinal dysfunction /unable to receive study medication
- Patients with a do not resuscitate (DNR) order /imminent death
- Not expected to survive initial 48 hours of admission or treatment withdrawal imminent within 24 hours.
- Hypercalcemia (> 2.65 mmol/l total calcium and/ or >1.35 mmol/l ionized calcium at screening)
- Known kidney stones, active tuberculosis or sarcoidosis (within the last 12 months)
- Pregnancy/lactation
- Hypersensitivity to drug or excipient

Randomisation and Blinding

Patients will be randomly assigned to either placebo or vitamin D3 in a 1:1 ratio, using the web-based randomisation service “Randomizer for Clinical Trials” developed at the Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz. Patients will be stratified according to trial site (ICU) and gender. An independent statistician will set up the study in the Randomizer.
The following method will be used to maintain the blind: the randomisation list from the randomizer.at will be kept strictly confidential and no routine vitamin D testing is done after

study inclusion. The independent statistician and unblinded pharmacist will keep treatment allocation information confidential until database lock.

In case of safety concerns (eg. severe hypercalcemia > 3.5 mmol/L), participants of the study may be unblinded by the local investigator at each participating site and/or the coordinating centre. This will be done and documented with the Randomizer.

Endpoints

Primary effectiveness outcome measure:

- All-cause mortality at 28 days after randomisation

Secondary effectiveness outcome measures:

- 90-day and 1-year all-cause mortality
- ICU and hospital mortality and length of stay
- Change in organ dysfunction on day 5 as measured by Sequential Organ Function Assessment score (SOFA), number of organ failures (0-6; as defined by > 2 SOFA points in each of the 6 categories)
- Hospital and ICU readmission until day 90
- Discharge destination (home, rehabilitation, other hospital)
- Katz Activities of Daily Life at day 90
- Self-reported infections requiring antibiotics until day 90

Safety outcomes:

- Hypercalcaemia at day 5
- Self-reported falls, fractures until day 90
- New episodes of kidney stones

In the UK arm, additional secondary outcomes are:

- Health-related quality of life (EQ-5D-5L) at 90 days and 1 year
- Disability assessment (WHO-DAS 2.0) at 90 days and 1 year.
- Secondary health care utilisation in the first year (ICU and hospital length of stay, readmissions and utilisation of hospital and community care resources after hospital discharge one year after randomisation), from Hospital Episode Statistics, civil registry data held by NHS Digital and patient questionnaires.
- Health economics analysis
    - cost effectiveness of screening for and treating VDD in critical illness
    - cost per quality-adjusted life year gained one year after randomisation and at end of life

Sample size considerations

The sample size for this multinational study is based on the primary endpoint 28-day mortality. In the VITdAL-ICU study, 28-day mortality rates of 36% (37/102) in the placebo group and 20% (20/98) in the vitamin D Group were observed [10]. The VITDALIZE study has been designed to be sufficiently powered to detect a smaller, but clinically relevant absolute mortality difference of 5% with a power of 80% with an assumed baseline mortality rate of 25%. This corresponds to a clinically highly relevant relative risk reduction of 20%. Multicentre trials generally have a smaller treatment effect than monocentre studies [14]. We assume that this will also be the case for the VITDALIZE study. Furthermore, our assumed 5% absolute mortality difference is in line with a recent survey among clinical intensivists that the largest median treatment effect considered plausible by intensivists for current ongoing ICU multicentre trials is 3 to 5% [15].

Using a fixed sample size design and a two-sided log-rank test for equality of survival curves with a two sided alpha level of 5%, a sample size of N=1,093 per group will be needed to achieve a power of 80% (total sample size of 2,186).

Incorporating one interim analysis after inclusion of 50% of the patients a total sample size of at least N=2,194 (494 events) is required to achieve 80% power using a O'Brien-Fleming spending function [16]. Accounting for a drop-out rate of approximately 10% yields a total sample size of N=2,400 patients. For sample size calculation the software package nQuery 7.0 +nTerim 2.0 was used.

Statistical analysis

The primary analysis will be performed on the intention-to-treat population (ITT). The ITT will include all patients who receive at least the loading dose of the study medication. All patients will be analysed according to the treatment assignment during randomisation. The per protocol population will include all patients who received the loading dose and have a compliance > 80%. Compliance is defined as self-reported percentage of doses ingested until day 90.

The safety analyses will be based on the treated set, which is defined as all randomised patients who receive at least one dose of trial medication. All patients will be analysed according to the treatment they received.

Data analysis

All clinical and safety data collected in the study will be analysed with SAS v9.4. Data will be presented as summary tables and, where appropriate, as plots. Continuous data will be described by means, standard deviations, medians and upper and lower quartiles unless

otherwise stated. The number of observations and minimum and maximum values are also included.
Categorical data will be summarized using frequencies and percentages.

The primary outcome, 28-day all-cause mortality, will be displayed using Kaplan Meier estimates of survival curves in each treatment arm. Group comparison will be made using a stratified two-sided log rank test. The unstratified log-rank test will be performed as a sensitivity analysis. The Cox regression model, including treatment and stratification factor, will be used to estimate the hazard ration and its 95% CI. Details will be defined in a Statistical Analysis Plan.

ICU, hospital mortality, 90-day mortality and 1-year mortality will be analysed as secondary outcomes using Kaplan Meier estimates of survival curves. For the other secondary parameters, comparison between groups will be performed using appropriate parametric or non-parametric methods and Chi-square tests.

The safety outcomes, (hypercalcemia on day 5, new kidney stones, self-reported falls, and fractures until day 90) will be analysed as binary variables and compared with Chi-square tests.

Demographic and Baseline Characteristics
In a summary of demographic, baseline and diagnostic characteristics (age, weight, height, sex, SAPS III, Charlson comorbidity index) a comparison of the treatment groups will be performed. To this end, appropriate descriptive and inferential statistics will be applied. Relevant medical history will be also displayed using summary statistics according to the two treatment groups.

Subgroup analysis
Predefined subgroup analyses will be performed for all primary and secondary outcomes based on the following group definitions as exploratory analysis:
• Kidney function (CKD 4 or lower vs. higher at inclusion)
• Sepsis (admission diagnosis) vs. non-sepsis defined by the 2016 criteria (suspected infection/ qSOFA on day 0 – respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less)

Missing data handling

All available data will be used in the analyses and data summaries. There will be no imputation of any missing data.

Planned interim analysis

The trial uses a group sequential design with one interim analysis when 50% of the planned enrolled patients in each arm (N=600 per arm) have completed their day 28 assessment by the independent data safety monitoring board. The enrolment of patients will continue while the interim analysis is performed. This interim analysis is intended to test for efficacy, i.e. the trial may be terminated after the interim analysis, if the main question can already be answered at this interim analysis. If the interim analysis shows a benefit for the vitamin D group, the DSMB may recommend early study termination. The interim analysis will be performed only for the primary outcome 28-day mortality. The O' Brien-Fleming rule will be used to stop the trial early for efficacy. In detail, if the p-value of the log rank test is smaller than 0.003, then the trial can be stopped early by the DSMB.

DISCUSSION

Vitamin D has much broader, pleiotropic effects that extend well beyond the musculoskeletal system [1]. Vitamin D regulates more than 1,000 genes and has an influence on muscles, blood vessels, cell proliferation and differentiation and is an important regulator of the immune system. Recent studies have demonstrated that low vitamin D levels are an independent risk factor for mortality in critically ill patients [17–22] reflecting the relevant role of vitamin D.

Worldwide, the prevalence of vitamin D deficiency in intensive care patients ranges between 40-70%. Therapeutic interventions like surgery, fluid resuscitation, extracorporeal membrane oxygenation, cardiopulmonary bypass, dialysis and plasma exchange and hepatic, parathyroid and renal dysfunction may significantly reduce vitamin D levels [23].

Pleiotropic effects of vitamin D on the immune system, glucose metabolism, and calcium homeostasis are essential in critically ill patients. Vitamin D deficiency carries an additional risk due to mortality and morbidity to these patients. ICU patients often suffer from immunological dysfunction and changes in body composition (loss of muscle mass, increase in the adipose tissue). Every additional day staying on ICU increases the chance of becoming dependent on care with prolonged rehabilitation and recovery time. Interventions, such as vitamin D supplementation, may also have the potential to improve health related quality of life.

Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating of vitamin D deficiency is currently not routinely performed.

The VITDALIZE trial is a large randomized, multicentre international study designed to demonstrate the clinical benefit of vitamin D supplementation in critically ill patients with severe vitamin D deficiency. The primary outcome will be 28-day all-cause mortality. All-cause mortality represents a “hard” endpoint that is not prone to measurements bias. Most importantly, the European Medicines Agency recently recommended short-term (28-day) all-cause mortality as the most relevant primary efficacy endpoint in confirmatory clinical trials assessing the efficacy of drugs or medicinal products in patients with life-threatening acute illnesses, e.g. in critically ill patients with sepsis [24] or with acute respiratory distress syndrome [25]. Long-term effects will be reflected by the secondary endpoints 90 days and 1-year mortality and the secondary endpoint Katz Activity of Life will reflect the health related quality of life. The UK arm will also be assessing the cost-effectiveness and health economics of the intervention.

The VIOLET trial is another important and similar, yet substantially different RCT that has stopped recruitment in July 2018 but no results have been published at the time of writing. VIOLET included patients with vitamin D deficiency (point-of-care test, < 20ng/ml) in patients at risk for ARDS, but not necessarily ICU patients. The intervention consisted of a single bolus loading dose (540,000 I.U. vitamin D3), but no maintenance dose; and the primary endpoint is 90 day mortality. Together, these two large trials in acutely ill patients will greatly advance our knowledge in this field.

As vitamin D3 application is a simple, low-cost, safe and well-tolerated intervention, it has great potential to improve survival and quality of life in critically ill patients and could be implemented worldwide immediately.

ETHICS and DISSEMINATION

Study protocol (V1.3, EudraCT-No. 2016-002460-13), patient information, and informed consent were approved by the Ethics Committee of the University of Graz (EK 1289/2016), and will be submitted to each participating trial centre.

Each patient must give written informed consent to participate in the study.

Recruitment in Austria started in October 2017 and in Belgium in January 2019. The current study protocol (V1.3) was released in January 2018. This trial was registered at http://clinicaltrials.gov (identifier NCT03188796) in June 2017. So far, more than 200 patients have been randomised in > 15 active centres. The planned recruitment lasts for approximately another 36 months. The United Kingdom has applied for funding at NIHR and likely will start recruitment in 2020. Germany will be funded by the BMBF (Federal Ministry of Education and Research) and will start recruitment in June 2019. The participation of Switzerland is planned, but will depend on funding possibilities.

Upon completion, results will be published in a peer-reviewed scientific journal.

List of abbreviations

MCT=Medium Chain Triglycerides; ICU=Intensive Care Unit; I.U.=International Units

REFERENCES

1. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab. 2009;94:26–34. doi:10.1210/jc.2008-1454.
2. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. BMJ. 2017;356:i6583. doi:10.1136/bmj.i6583.
3. Amrein K, Papinutti A, Mathew E, Vila G, Parekh D. Vitamin D and critical illness: What endocrinology can learn from intensive care and vice versa. Endocr Connect. 2018;7:R304-R315. doi:10.1530/EC-18-0184.
4. Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. J Autoimmun 2017. doi:10.1016/j.jaut.2017.07.007.
5. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2014:CD007470. doi:10.1002/14651858.CD007470.pub3.
6. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, et al. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin D trials. J Clin Endocrinol Metab. 2012;97:2670–81. doi:10.1210/jc.2011-3328.
7. Aspelund T, Grübler MR, Smith AV, Gudmundsson EF, Keppel M, Cotch MF, et al. Effect of Genetically Low 25-Hydroxyvitamin D on Mortality Risk: Mendelian Randomization Analysis in 3 Large European Cohorts. Nutrients 2019. doi:10.3390/nu11010074.
8. Amrein K, Litonjua AA, Moromizato T, Quraishi SA, Gibbons FK, Pieber TR, et al. Increases in pre-hospitalization serum 25(OH)D concentrations are associated with improved 30-day mortality after hospital admission: A cohort study. Clin Nutr. 2016;35:514–21. doi:10.1016/j.clnu.2015.03.020.
9. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. Public Health Nutr. 2011;14:938–9. doi:10.1017/S1368980011000565.
10. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, 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;312:1520–30. doi:10.1001/jama.2014.13204.
11. Manson JE, Cook NR, Lee I-M, Christen W, Bassuk SS, Mora S, et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. N Engl J Med. 2019;380:33–44. doi:10.1056/NEJMoa1809944.
12. Leclair TR, Zakai N, Bunn JY, Gianni M, Heyland DK, Ardren SS, Stapleton RD. Vitamin D Supplementation in Mechanically Ventilated Patients in the Medical Intensive Care Unit. JPEN J Parenter Enteral Nutr 2019. doi:10.1002/jpen.1520.

13. Langlois PL, Szwec C, D'Aragon F, Heyland DK, Manzanares W. Vitamin D supplementation in the critically ill: A systematic review and meta-analysis. Clin Nutr 2017. doi:10.1016/j.clnu.2017.05.006.
14. Dechartres A, Boutron I, Trinquart L, Charles P, Ravaud P. Single-center trials show larger treatment effects than multicenter trials: evidence from a meta-epidemiologic study. Ann Intern Med. 2011;155:39–51. doi:10.7326/0003-4819-155-1-201107050-00006.
15. Ridgeon EE, Young PJ, Bellomo R, Mucchetti M, Lembo R, Landoni G. The Fragility Index in Multicenter Randomized Controlled Critical Care Trials. Crit Care Med. 2016;44:1278–84. doi:10.1097/CCM.0000000000001670.
16. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979;35:549–56.
17. 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. doi:10.1097/CCM.0b013e31822d74f3.
18. Moraes RB, Friedman G, Wawrzeniak IC, Marques LS, Nagel FM, Lisboa TC, Czepielewski MA. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics (Sao Paulo). 2015;70:326–32. doi:10.6061/clinics/2015(05)04.
19. 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. doi:10.1186/cc13790.
20. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360:1912–4. doi:10.1056/NEJMc0809996.
21. Venkatram S, Chilimuri S, Adrish M, Salako A, Patel M, Diaz-Fuentes G. Vitamin D deficiency is associated with mortality in the medical intensive care unit. Crit Care. 2011;15:R292. doi:10.1186/cc10585.
22. Arnson Y, Gringauz I, Itzhaky D, Amital H. Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. QJM. 2012;105:633–9. doi:10.1093/qjmed/hcs014.
23. McNally JD, Menon K. Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance. Transl Pediatr. 2013;2:99–111. doi:10.3978/j.issn.2224-4336.2013.07.03.
24. Clinical investigation of medicinal products for the treatment of sepsis. Reference CPMP/EWP/4713/2003.
25. Clinical investigation of medicinal products in the treatment of patients with acute respiratory distress syndrome. Reference CPMP/EWP/504/97 Rev.1.

AUTHOR'S CONTRIBUTION

All authors have substantially contributed to the interpretation of current specific knowledge, which resulted in the conception and design of the present trial. KA, DP, SW, JCP, PE, PS, DT and PM are investigators of the present trial and participated in the acquisition of funding and contribute to the collection of data. AB and RR wrote the statistical methods. All authors have been involved in drafting the manuscript or revising it critically for important intellectual content, and approved the final manuscript.

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PATIENT and PUBLIC INVOLVEMENT STATEMENT

We did not involve patients or the public in our work.

WORD COUNT

3,395 words

ADDITIONAL MATERIAL

Study protocol, version 1.3 from 24th January 2018

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+The VITDALIZE collaboration group will be listed as an author, and the names of the individual members of the group will be searchable through their individual PubMed records:

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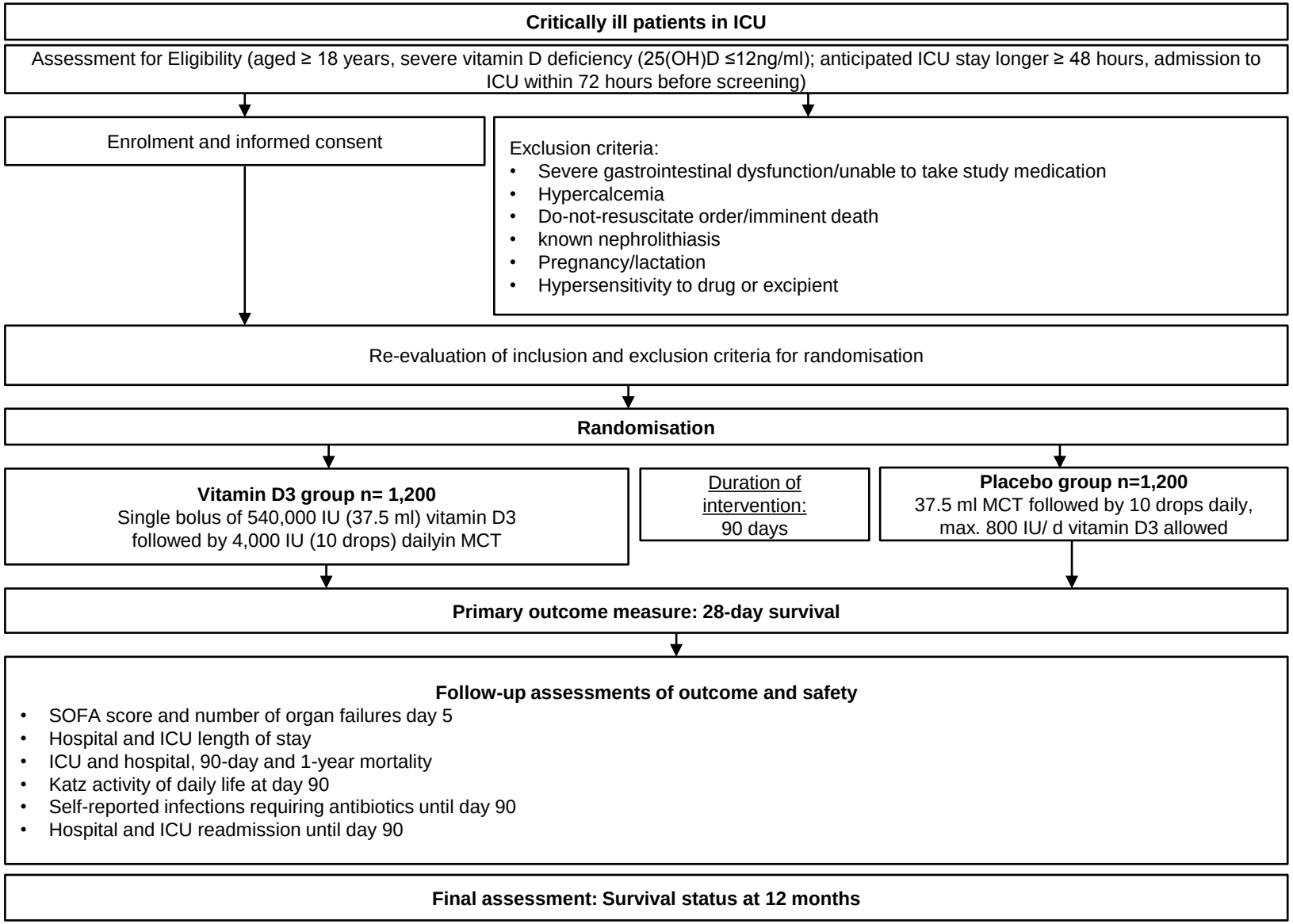
Critically ill patients in ICU
Assessment for Eligibility (aged ≥ 18 years, severe vitamin D deficiency (25(OH)D ≤12ng/ml); anticipated ICU stay longer ≥ 48 hours, admission to ICU within 72 hours before screening)
Enrolment and informed consent
Exclusion criteria:
• Severe gastrointestinal dysfunction/unable to take study medication
• Hypercalcemia
• Do-not-resuscitate order/imminent death
• known nephrolithiasis
• Pregnancy/lactation
• Hypersensitivity to drug or excipient
Re-evaluation of inclusion and exclusion criteria for randomisation
Randomisation
Vitamin D3 group n= 1,200
Single bolus of 540,000 IU (37.5 ml) vitamin D3 followed by 4,000 IU (10 drops) dailyin MCT
Duration of intervention: 90 days
Placebo group n=1,200
37.5 ml MCT followed by 10 drops daily, max. 800 IU/ d vitamin D3 allowed
Primary outcome measure: 28-day survival
Follow-up assessments of outcome and safety
• SOFA score and number of organ failures day 5
• Hospital and ICU length of stay
• ICU and hospital, 90-day and 1-year mortality
• Katz activity of daily life at day 90
• Self-reported infections requiring antibiotics until day 90
• Hospital and ICU readmission until day 90
Final assessment: Survival status at 12 months

PROTOCOL

The VITDALIZE Study

Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicenter, placebo-controlled double-blind phase III RCT

EudraCT number 2016-002460-13

PRINCIPAL INVESTIGATOR

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SYNOPSIS

STUDY NAME	The VITDALIZE Study: Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicenter, placebo-controlled double-blind phase III RCT
SPONSOR	Medical University of Graz Auenbruggerplatz 15 8036 Graz, Austria
STEERING COMMITTEE	Consists of the coordinating investigator and co-investigators
EARLIEST START DATE	Q2 2017
RECRUITMENT PHASE	2-3 years
FOLLOW UP	12 months
STUDY CENTER	Ca. 30 (ca. 15 non-academic)
PARTICIPATING COUNTRIES	Austria Germany Switzerland/UK/Belgium
RATIONALE & BACKGROUND	In the VITdAL-ICU trial using a large oral dose of vitamin D3 in 480 adult critically ill patients, there was no benefit **regarding the primary endpoint hospital length of stay. However, the predefined subgroup with severe vitamin D deficiency (25(OH)D ≤ 12ng/ml) had significantly lower 28-day mortality** (36.3% placebo vs. 20.4% vitamin D group, HR 0.52 (0.30-0.89), **number needed to treat = 6**). Therefore, high-dose vitamin D3 in a population of severely vitamin D deficient critically ill patients is a promising and inexpensive intervention that requires confirmatory multicenter studies. To date, **only 7 interventions (e.g. noninvasive ventilation or prone positioning) have ever demonstrated mortality benefit for ICU patients in multicenter trials.** In case of benefit, vitamin D treatment in critically ill patients could be immediately implemented worldwide.
TARGET NUMBER OF PATIENTS TO BE INCLUDED	Maximum 2400 patients (1200 per group) 1 interim analysis after 1200 patients

	The sample size is based on an anticipated 5% absolute mortality reduction assuming an overall 28-mortality of 25% in the placebo group
INTERVENTION	Cholecalciferol (vitamin D3) versus placebo: Day 0: One single bolus loading dose 540,000 IU of oral (or enteral) vitamin D3 followed by 4000 IU of vitamin D3 daily for the entire active study period (90 days) versus placebo (medium chain triglycerides, MCT) – total dose 900,000 IU vitamin D3
INCLUSION CRITERIA	- ≥18 years - Anticipated ICU stay ≥ 48 hours - Admission to ICU ≤ 72 hours before screening - Severe vitamin D deficiency (≤12 ng/ml or undetectable)
EXCLUSION CRITERIA	- Severe gastrointestinal dysfunction (> 400 ml residual volume)/unable to take study medication - DNR order/imminent death - hypercalcemia - known nephrolithiasis, active tuberculosis or sarcoidosis (within the last 12 months) - pregnancy/lactation - not deemed appropriate by study team/physician - hypersensitivity to drug or excipient
AIM OF THE TRIAL	To test if high-dose vitamin D3 is beneficial for the clinical outcome of adult critically ill adult patients with severe vitamin D deficiency
PRIMARY OUTCOME	**28-day mortality**
SECONDARY/SAFETY OUTCOMES	90-day mortality 1-year mortality ICU and hospital mortality Hospital and ICU length of stay SOFA Score at day 5 (48 hours tolerance) and number of organ failures (> 2 SOFA points in each of the 6 categories) Katz Activities of Daily Life (ADL) at day 90 Self - reported infections requiring antibiotics until day 90

	Hospital and ICU readmission until day 90 Discharge disposition Microbiome analysis day 0 and day 5 (optional, for Medical University Graz centers only) Laboratory:25OHD and 1,25OH2D at day 1 and 5 (optional, for Medical University Graz centers only) Safety outcomes: Hypercalcemia on day 5 (48 hours tolerance) Self reported falls, fractures until day 90 New episodes of kidney stones
FOLLOW UP PROCEDURE	by telephone, 3 months, by telephone, 12 months (mortality only)
RANDOMIZATION	randomizer.at stratification by ICU and gender
ETHICS COMMITTEE INFORMED CONSENT	Austria: Deferred/surrogate informed consent (IC) Germany: urgent approval of one legal substitute by court, surrogate IC by legal substitute, deferred IC Switzerland: Deferred IC, relatives and unrelated physician
ESTIMATED RECRUITMENT RATES	20-100 per center and year
DATA SAFETY MONITORING BOARD	Peter Suter, Geneva Heike Bischoff-Ferrari, Zurich Martin Posch, Vienna
TIMELINE	Submitted to ESICM Clinical Trials Group 12/2015 Submitted to Ethical Committee Graz Q3/2016 Submitted to Ministry of Health Q4/2016 Funding sources (KLIF) Q3 /2017 Recruitment Start Run-In-Phase Q4/2017 Rollout 2018-2021

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See separate file

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH guidelines.

Sponsor :

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Statistician/s responsible for biometry

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Title: PhD, Professor

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

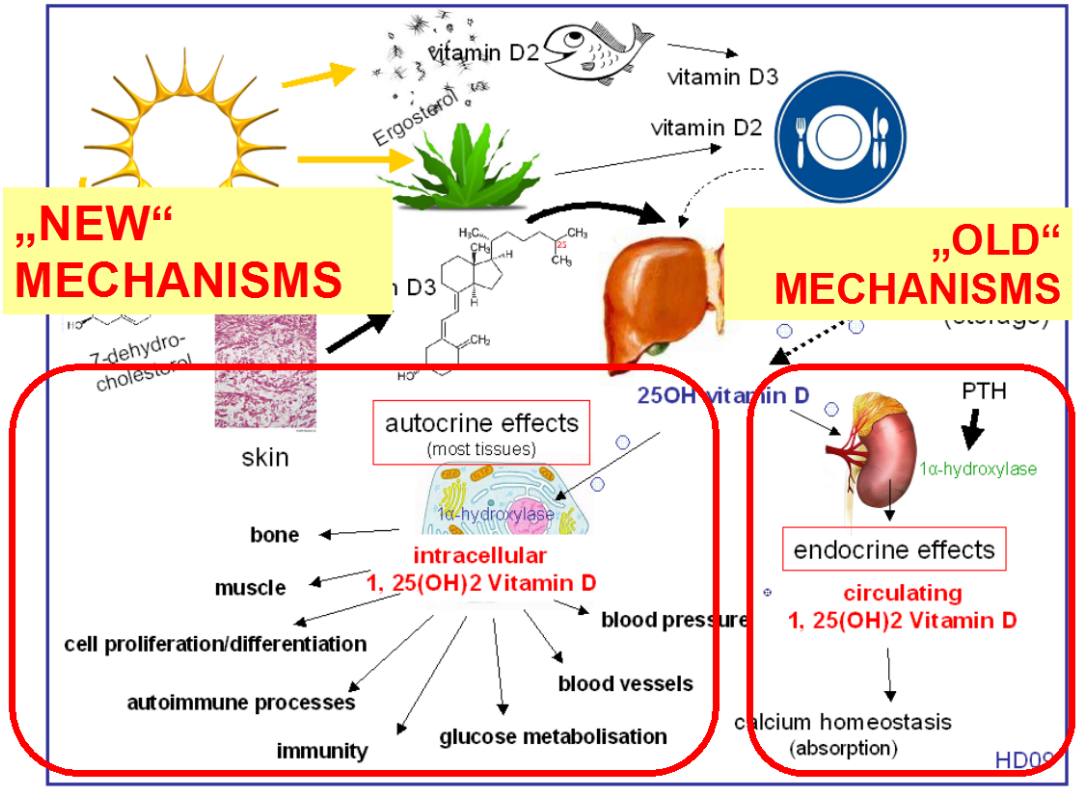
Name:

Title:

BACKGROUND

Traditionally, vitamin D has been thought to be primarily important to bone health, and its most severe form, rickets in children, seems to support this notion. However, in the last decade, vitamin D has seen an unprecedented revival, and currently several thousands of papers are published annually. This renewed interest was sparked by the finding that vitamin D has much broader, pleiotropic effects that extend well beyond the muskuloskeletal system (1) (overview in Figure 1, Dobnig H.). It is now estimated that vitamin D, which in reality is a precursor to a steroid hormone with a specific nuclear receptor (vitamin D receptor, VDR), regulates more than 200 genes and is also an important regulator of the immune system. Many of these findings have been studied in the vitamin D knockout mouse which has a variety of severe health problems and ages faster than wild-type mice (2). Vitamin D deficiency is more likely in patients with chronic diseases and limited mobility, but there is strong biological plausibility that supports a contributing role of vitamin D deficiency to poor outcomes.

The nuclear vitamin D receptor (VDR) is widely present in different cell types and organs that are relevant to critically ill patients via genomic and nongenomic pathways (2). The most important effects on target organs that are relevant during and after critical illness, namely muscle, heart, immune function, kidney and bone are summarized in the Figure.



Vitamin D needs are usually met with UV-B exposure from sunlight (or supplements), because

very few foods are rich in vitamin D (3). Endogenous vitamin D production is influenced by age, season, latitude and skin color. During the winter months, it is compromised at latitudes above 35°. During winter in the higher latitudes, sunlight has a longer tangential path to reach the earth's surface, resulting in the absorption and loss of the UV-B photons in the ozone stratosphere.

25(OH)D is the major circulating vitamin D metabolite, and its measure best reflects an individual's vitamin D status (4). Recently, there is a debate whether free vitamin D may be a better marker as there seem to be large interindividual differences (5), however the assay is currently not widely available.

Unfortunately, the enthusiasm is currently not supported by unequivocal clinical data, which is explainable by the small number of methodologically sound vitamin D intervention trials targeting patients with proven vitamin D deficiency at risk for high mortality and morbidity, similar to suboptimal clinical studies studying other nutrients (Heaney 2013). On the other hand, as opposed to many other interventions, vitamin D3 (cholecalciferol) has been shown to improve survival in a 2011 and a 2014 Cochrane metaanalysis (6).

The *link between vitamin D and critical illness is new (2009),* but intriguing, because

1) the majority of critically ill patients is vitamin D deficient,
2) standard care currently gives little or no vitamin D and
3) critically ill patients have a very high risk for mortality and morbidity.

VITAMIN D AND MORTALITY

At first glance, it seems absurd that one single substance should have such a profound effect. However, vitamin D deficiency causes skeletal and nonskeletal disorders in adults and children and seems to predispose to a variety of respiratory, immune, infectious, neurologic, cardiovascular and other diseases (3). There is also a strong association between a poor vitamin D status and excess morbidity and mortality in the general population, but also in critical illness, both in children and in adults (3, 7, 8).

Mendelian randomization studies

In 2014, the largest analysis (n>96,000) to date was published by Afzal et al. and evaluated overall mortality, cancer mortality and other mortalities (9). The odds ratio for a genetically determined 20 nmol/L lower plasma 25-hydroxyvitamin D concentration was 1.30 (1.05 to 1.61) for all cause mortality, with a corresponding observational multivariable adjusted odds ratio of 1.21 (1.11 to 1.31). Corresponding genetic and observational odds ratios were 0.77 (0.55 to 1.08) and 1.13 (1.03 to 1.24) for cardiovascular mortality, 1.43 (1.02 to 1.99) and 1.10

(1.02 to 1.19) for cancer mortality, and 1.44 (1.01 to 2.04) and 1.17 (1.06 to 1.29) for other mortality. The results were robust in sensitivity analyses. Each increase in DHCR7/CYP2R1 allele score was associated with a 1.9 nmol/L lower plasma 25-hydroxyvitamin D concentration and with increased all cause, cancer, and other mortality but not with cardiovascular mortality.

Cochrane metanalyses

Two consecutive Cochrane metaanalyses by Goran Bjelakovic with > 90,000 participants (2011 and 2014) showed that vitamin D3 (not other forms like vitamin D2 or active metabolites) supplementation was linked to significantly improved survival. Trial sequential analysis suggested a number needed to treat between 150 (2014) and 161 to prevent one additional death (2011; RR in both analyses 0.94, 95% CI 0.91 to 0.98). It has to be noted that most of the participants were elderly women > 70 years and only a small minority of the studies included patients with specific diseases (6, 10). In the 2014 analysis, vitamin D3 also statistically significantly decreased cancer mortality (RR 0.88; 95% CI 0.78 to 0.98).

Individual patient data (IPD) analysis

Lars Rejnmark published an IPD analysis using data fom 8 major vitamin D trials in 2012. The > 70,000 participants were mostly female (87%) and had a median age of 70 years. Mortality was reduced by vitamin D with calcium by 9% (HR 0.91, 95% CI 0.84-0.98), corresponding to a NNT of 151 (11).

Other, more critical reports

In a 2013 systematic review, Autier et al. came to the conclusion that “low 25(OH)D is a marker of ill health” but “an exception would be slight gains in survival after the restoration of vitamin D deficits” (12).

In a 2014 publication, Bolland et al. undertook a trial sequential meta-analysis of randomized controlled trials using vitamin D, with or without calcium, to investigate the effects of vitamin D supplementation on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, cancer, total fracture, hip fracture, and mortality predefining a risk reduction threshold of 5% for mortality and 15% for other endpoints in unselected community-dwelling individuals (13). They concluded that overall, vitamin D supplementation did not reduce these outcomes, although it reduced hip fracture in institutionalised individuals when co-administered with calcium and there was „uncertainty as to whether vitamin D with or without calcium reduces the risk of death“. Specifically, “vitamin D with or without calcium reduced the risk of death by 4% in traditional meta- analyses, but trial sequential analysis suggested that uncertainty remains in this finding“.

Vitamin D and hospital mortality

In our own observational dataset (n=655), all-cause hospital mortality was significantly higher in patients with vitamin D deficiency compared to patients with sufficient levels. In adjusted Cox regression analysis, compared to normal vitamin D levels, in vitamin deficiency the HR for hospital mortality was 1.63, 95% CI 0.93 to 2.86, and in vitamin D insufficiency 1.01 (95% CI: 0.52 to 1.96) (14).

A recent metaanalysis on hospital mortality in critically ill patients showed a significant association of vitamin D deficiency and increased hospital mortality (OR 1.76; 95% CI, 1.38 to 2.24; P <0.001) (15).

VITAMIN AND SEPSIS

Vitamin D deficiency is associated with an increased susceptibility of sepsis (16, 17). Sepsis is one the most common reasons for ICU admission and nosocomial infections frequently complicate and prolong ICU stay in other patients. The incidence of sepsis continues to rise and is the leading cause of death in critically ill patients, affecting millions of patients annually worldwide with a mortality rate of approximately 25% (18).

Sepsis incidence and mortality is also higher during the winter months when 25(OH)D concentrations are lower (19). In patients with pneumonia, vitamin D deficiency is associated with an increased risk of ICU admission and mortality (20).

The link between vitamin D status and sepsis is biologically plausible because vitamin D has important pleiotropic effects on the immune system (21, 22). Vitamin D metabolizing enzymes and vitamin D receptors are present in many cell types including various immune cells such as antigen-presenting-cells, T cells, B cells and monocytes. It regulates both the innate and the adaptive immune system and seems to increase antimicrobial peptides (cathelicidin, or LL-37 in its active form; and β-defensin), which are present in many epithelia of the human body, including the respiratory system and the urogenital tract.

A recent observational study in 107 Italian sepsis patients showed that 25(OH)D levels < 7ng/ml on admission were a major determinant of clinical outcome (23). Benefits of VD replacement therapy in this population should be elucidated.

VITAMIN D AND CRITICAL ILLNESS

Critical illness did not really exist until the accidental discovery of penicillin and the invention of the first simple ventilator, the iron lung (both between World War I and II). Even then, however, the use of both antibiotics and respirators was greatly limited by availability and the

absence of dedicated intensive care units. During the 1952 poliomyelitis outbreak in Denmark, > 2700, often young and previously healthy patients became ill within a few months, and > 300 needed respiratory support, which called for a different strategy. The anesthesiologist Dr. Ibsen then established positive pressure ventilation by tracheal intubation, and 200 medical students were deployed to manually ventilate the patients. At this time, the first positive pressure volume controlled ventilators were developped and eventually replaced the medical students. Subsequently, mortality declined from 90% to around 25%. Patients were treated in three special 35-bed units, the precursors to modern intensive care units (ICU). The first Critical Care Residency was established at the University of Pittsburgh in 1962 and the Society of Critical Care Medicine was founded in 1970.

Widespread vitamin D deficiency is probably also of relatively recent origin, after the industrialization substantially changed our lifestyle with air pollution, urbanization and decreased time spent outdoors (24). The invention of the modern sitting office workplace in the 1940s likely contributes to this development, as not few individuals spend up to 15 hours per day sitting and the time spent outdoors is very limited (25).

Prevalence of vitamin D deficiency in the ICU

The prevalence of vitamin D deficiency in intensive care ranges typically between 40 and 70 % (8, 26-30). There are many reasons to be or become deficient in the ICU. Moreover, therapeutic interventions like surgery, fluid resuscitation, extracorporeal membrane oxygenation, cardiopulmonary bypass, dialysis and plasma exchange may significantly reduce vitamin D levels (31). Hepatic, parathyroid and renal dysfunction also places ICU patients at risk for disruption of vitamin D metabolism.

In a 2009 letter to the New England Journal of Medicine, Paul Lee was the first to publish that vitamin D deficiency in the ICU is a common problem based on data from 42 patients referred for endocrinological evaluation (32). This finding has been replicated and extended (33, 34). There are now many observational studies that consistently show an association between a poor vitamin D status and poor clinical outcomes (8, 26, 27, 34).

It is now clear that in critical illness

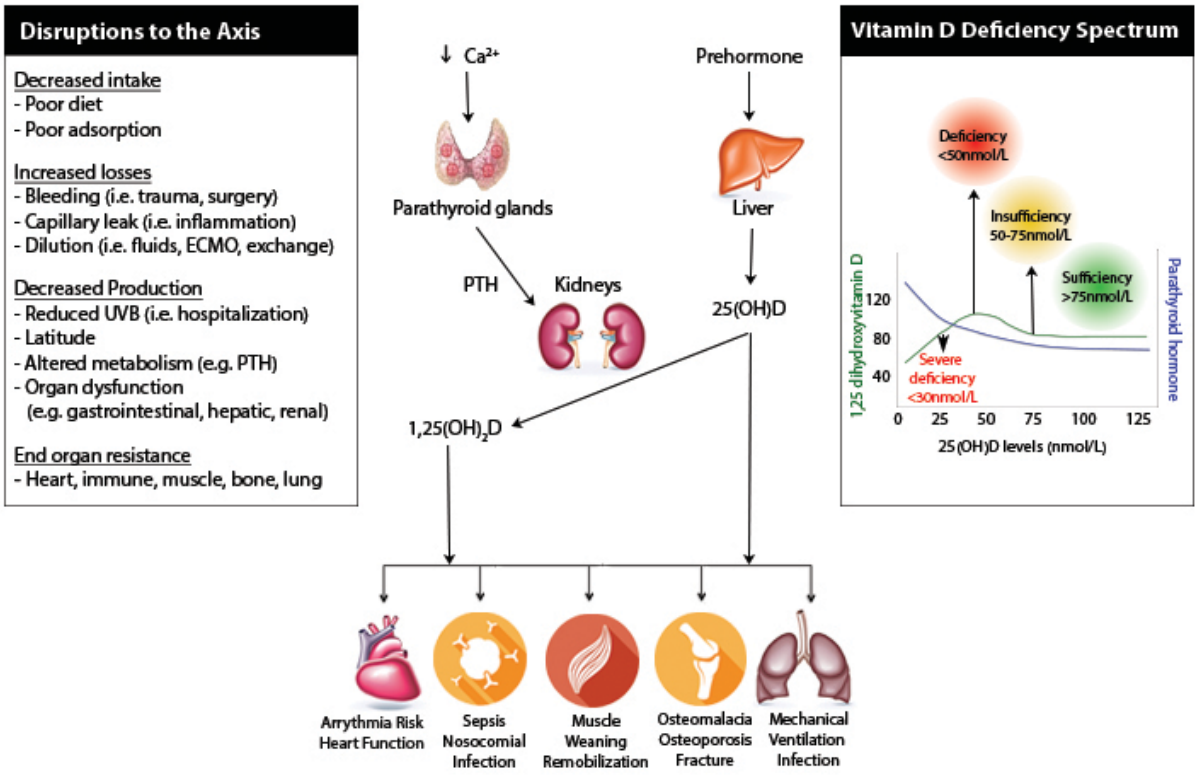
- the prevalence of vitamin D deficiency in ICU is very high
- vitamin D deficiency is associated with
    - excess morbidity
        - acute kidney injury (35)
        - acute respiratory failure, duration of mechanical ventilation and ARDS (36, 37)
        - sepsis, infections, positive blood cultures (28, 38-40)
    - excess mortality (adults and children) (14, 28, 41-44)

In summary, it may well be the case that vitamin D deficiency is indeed an "invisible accomplice to morbidity and mortality", as proposed earlier by Paul Lee (45).

Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating vitamin D deficiency is currently not routinely performed.

Vitamin D metabolism is disrupted in many ICU patients (overview in Figure below; reproduced from (33)). Additionally, many – especially medical – ICU patients enter the ICU in a deficient state because of preexisting poor lifestyle including malnutrition and preexisting disease.

The current ESPEN Guideline for parenteral nutrition in intensive care recommends All PN prescriptions should include a daily dose of multivitamins and of trace elements. (Grade C). The available parenteral (and enteral) multivitamin preparations contain very low doses, typically 200-250 IU per vial/table. Unfortunately, no intravenous high-dose vitamin D preparation is commercially available, although this is clearly needed, especially in patients with gastrointestinal malfunction, a common problem in critical illness (46).



Diagnosis of vitamin D deficiency in the ICU

In the general population, serum 25(OH)D is the generally accepted marker for determination of vitamin D status, however several issues regarding timing of the blood sample, assay specifics and the choice of metabolite are discussed (4, 47). Thresholds and terminology vary, but the most widely used definitions are as follows (to convert nmol/L to ng/ml, divide by 2.5) (3, 48):

	IOM Report 2011	**Holick M., NEJM 2007**
Vitamin D deficiency	< 12ng/ml	< 20ng/ml
Vitamin D sufficiency	> 20ng/ml	> 30ng/ml
Intoxication/potentially harmful	> 50ng/ml	>149ng/ml

These thresholds are based on biochemical indicators of axis stress and values below which the incidence for calcium malabsorption, secondary hyperparathyroidism and skeletal manifestation rises (49)**.**

30 nmol/l (=12ng/ml) has been a cut point below which individuals were considered to have a risk of "vitamin D deficiency" and was utilized in many publications as well as in the full version of the "Institute of Medicine's 2011 Dietary Reference Intakes" report. 25(OH)D levels below 12 ng/ml (or 30 nmol/l) hallmarked an increased risk for rickets, osteomalacia and decreased fractional calcium absorption. The following summary and figure is given on p. 368: *"The congruence of the data links serum 25(OH)D levels below 30 nmol/L with the following outcomes: increased risk of rickets, impaired fractional calcium absorption, and decreased bone mineral content (BMC) in children and adolescents; increased risk of osteomalacia and impaired fetal skeletal outcomes; impaired fractional calcium absorption and an increased risk of osteomalacia in young and middle-aged adults; and impaired fractional calcium absorption and fracture risk in older adults".* Furthermore, on page 370, the following statement is given: *"The lower end of the requirement range is consistent with 30 nmol/L, and deficiency symptoms may appear at levels less than 30 nmol/L depending upon a range of factors".*

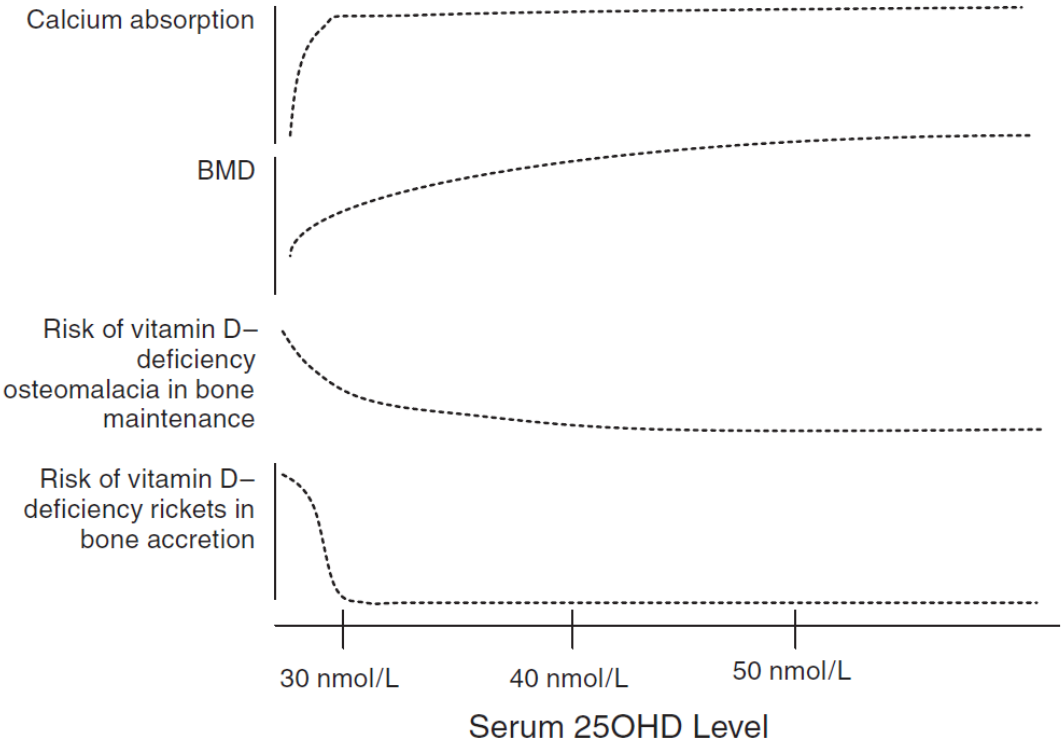


FIGURE 5-1 Conceptualization of integrated bone health outcomes and vitamin D exposure.

Current routine for vitamin D testing and supplementation in the ICU

In the general population, it is recommended that all healthy children and adults meet a daily minimum of vitamin D - the Institute of Medicine (IOM) recommends 400 to 800 IU of native vitamin D (48). The Endocrine Society recommends 1500 to 2000 IU for adult patients "at risk" for vitamin D deficiency (50).

In critical illness however, no standard of care has been established. Typical enteral and/or parenteral nutrition formulas supply ~ 200 -400 (<1000 IU) per day. In healthy individuals, such doses can prevent or improve vitamin D deficiency, but this requires months.

Rapid improvement of vitamin D status - justification of a high-dose vitamin D3 bolus

In hospitalized patients on low-dose vitamin D provided by nutrition formulas, 25(OH)D levels either remain constant or fall over time (51, 52). In critical illness, this approach does not substantially affect vitamin D levels in a meaningful time frame. Therefore, a bolus dose is an attractive option to rapidly improve vitamin D levels. The relatively long half-life of 25(OH)D following oral cholecalciferol supplementation allows for large loading doses of vitamin D3 which also have been shown to rapidly and safely normalize 25(OH)D levels in elderly patients

with vitamin D deficiency (53). Smaller doses of vitamin D3 are similarly or more effective but require a much longer period until a plateau of 25(OH)D levels can be reached.

In addition to the recommended daily allowance (RDA), an age specific daily upper tolerable intake levels of vitamin D has been suggested (1000 to 10,000 IU) (49, 50). The IOM uses a safety factor of 2.5, thus considering a permanent daily dose of 4000 IU of vitamin D to be safe (48).
The VITdAL-ICU study, the only phase III study evaluating rapid correction of vitamin D deficiency, provided a single enteral 540 000 IU loading dose of cholecalciferol to 237 critically ill adults. This dose increased 25(OH)D from 13 to 35 ng/ml until day 3 and did not provide evidence of safety concerns (51). Only very few patients achieved a 25(OH)D level of > 50ng/ml at any time point.

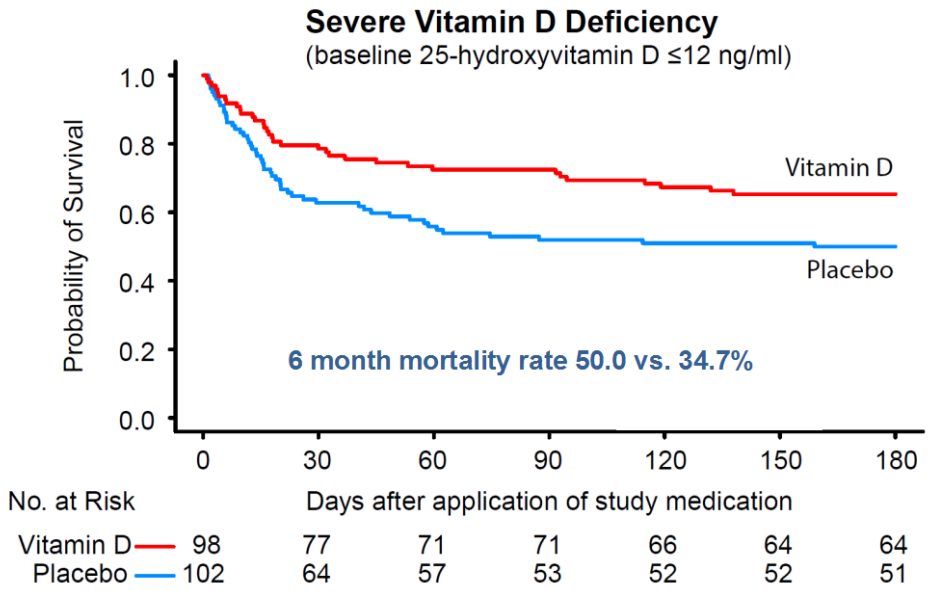
Supraphysiologic levels are not necessary, and there is evidence for a U-shape between 25(OH)D levels and outcomes (44). Vieth concluded in a review on the issue of "vitamin D toxicity" that only prolonged intakes of vitamin D at doses of >10,000 to 40,000 IU/day and 25(OH)D levels >200 ng/ml were shown to be associated with hypercalcemia (54). The daily follow-up dose of 4000 IU used in this study corresponds to the upper limit recommendation by the IOM (48).

Vitamin D intervention trials in the ICU

A very limited number of intervention trials, most including less than 30 patients, have been published. The only phase III study, our VITdAL-ICU study recruited from 2010 to 2012 and (n=475) did not find a difference in the primary endpoint „length of hospital stay" between placebo and high-dose vitamin D3. However, there was a non-significant absolute risk reduction in all-cause hospital mortality in the total population. The difference was larger (17.5%) and significant in the predefined subgroup of patients with severe vitamin D deficiency at baseline, see Kaplan Meier curve below (n=200, 28.6 vs 46.1%, p=0.01, 0.56 (0.35-0.90)), corresponding to a number needed to treat of 6. (51)
As this was only a secondary endpoint in the predefined subgroup with severe vitamin D deficiency, this finding is hypothesis generating and requires further study, leading to this application.
In our study, we were unable to identify a mechanism by which this benefit was achieved. Interestingly, looking at the causes of death, the vitamin D group seemed to benefit in every category (see below).

Table 2. Length of Stay and Mortality Outcomes for the Total and Subgroup Populations

	Total Study Population (N = 475)			Prespecified Subgroup Population					
				Severe Vitamin D Deficiency[a] (n = 200)			Less-Severe Vitamin D Deficiency[b] (n = 275)		
	Placebo (n = 238)	Vitamin D_3 (n = 237)	*P* Value	Placebo (n = 102)	Vitamin D_3 (n = 98)	*P* Value	Placebo (n = 136)	Vitamin D_3 (n = 139)	*P* Value
Length of stay, median (range)									
Hospital, d[c]	19.3 (0.1-154.1)	20.1 (0.2-181)	.98	19.0 (1.0-154.1)	20.1 (0.2-181)	.40	20.5 (0.1-113.9)	20.1 (0.2-133)	.47
ICU, d	10.7 (0.1-154.1)	9.6 (0.2-181)	.38	9.1 (0.8-154.1)	9.7 (0.2-181)	.98	12.3 (0.1-113.9)	9.0 (0.2-127)	.26
Mortality, No. (%)									
ICU	63 (26.5)	54 (22.8)		34 (33.3)	23 (23.5)		29 (21.3)	31 (22.3)	
HR (95% CI)	0.97 (0.67-1.39)		.86	0.70 (0.41-1.19)		.18[d]	1.32 (0.79-2.20)		.28[d]
28-d	68 (28.6)	52 (21.9)		37 (36.3)	20 (20.4)		31 (22.8)	32 (23.0)	
HR (95% CI)	0.76 (0.53-1.09)		.14	0.52 (0.30-0.89)		.02[d]	1.06 (0.64-1.73)		.83[d]
Hospital	84 (35.3)	67 (28.3)		47 (46.1)	28 (28.6)		37 (27.2)	39 (28.1)	
HR (95% CI)	0.81 (0.58-1.11)		.18	0.56 (0.35-0.90)		.01[d]	1.12 (0.72-1.77)		.61[d]
6-mo	102 (42.9)	83 (35.0)		51 (50.0)	34 (34.7)		51 (37.5)	49 (35.3)	
HR (95% CI)	0.78 (0.58-1.04)		.09	0.60 (0.39-0.93)		.02[d]	0.95 (0.64-1.41)		.81[d]
Causes of death, No. (%)									
Sepsis	30 (29.4)	26 (31.3)		16 (31.4)	12 (25.3)		14 (27.5)	14 (28.6)	
Cardiovascular	30 (29.4)	24 (28.9)	.99	13 (25.5)	9 (26.5)	.95	17 (33.3)	15 (30.6)	.98
Neurologic	19 (18.6)	14 (16.9)		8 (15.7)	4 (11.8)		11 (21.6)	10 (20.4)	
Other	23 (22.5)	19 (22.9)		14 (27.5)	9 (26.5)		9 (17.6)	10 (20.4)	



To date, only 7 interventions have ever demonstrated a mortality benefit for ICU patients in multicenter trials (see Table below, e.g. noninvasive ventilation or prone positioning), often only in relatively small subgroups such as resuscitated patients or in ARDS (55). NNTs ranged between 3 and 11 with the exception of tranexamic acid in trauma patients. In case of similar benefit, vitamin D treatment in critically ill patients could be immediately implemented worldwide.

Treatment	Centers	Patients	p	Absolute Risk Reduction	Relative Risk Reduction	Number Need to Treat to Save One Life	Follow-Up	Stopped at Interim Analysis	Blinding
Albumin in hepatorenal syndrome (3)	7	126	0.01	0.191	0.668	5	Hospital discharge*; 90 d*	No	Yes
Daily interruption of sedatives (17)	4	336	0.01	0.134	0.232	7	28 d; 1 yr*	No	No
Mild hypothermia (4)	9	275	0.02	0.142	0.258	7	Hospital discharge, 6 mo*	No	No
Noninvasive ventilation (5)	5	85	0.02	0.193	0.675	5	Hospital discharge*	No	No
Noninvasive ventilation (18)	3	50	0.009	0.2	0.714	5	60 d*	No	No
Noninvasive ventilation (19)	14	236	0.05	0.101	0.498	10	Hospital discharge*	No	No
Noninvasive ventilation (20)	3	105	0.028	0.213	0.548	5	ICU discharge*; 90 d*	No	No
Noninvasive ventilation (21)	2	162	0.025	0.142	0.871	8	ICU discharge*; hospital discharge; 90 d*	No	No
Noninvasive ventilation (22)	11	90	0.015	0.12	0.828	7	Hospital discharge*	No	No
Noninvasive ventilation (23)	3	106	0.0244	0.197	0.64	5	ICU discharge; hospital discharge; 90 d*	No	No
Noninvasive ventilation (24)	3	82	0.014	0.122	0.836	8	Hospital discharge*; 6 mo*; 1 yr*	No	No
Prone position (6)	27	474	< 0.001	0.168	0.512	6	28 d; 90 d*	No	No
Protective ventilation (7)	2	53	< 0.001	0.329	0.465	3	ICU discharge*; hospital discharge; 28 d*	Yes	No
Protective ventilation (8)	10	861	0.007	0.088	0.222	11	Hospital discharge*	Yes	No
Protective ventilation (25)	8	103	0.017	0.238	0.441	4	ICU discharge*; hospital discharge*; 28 d*	Yes	No
Tranexamic acid (26)	247	20,211	0.0035	0.015	0.094	68	Hospital discharge*	No	Yes

*Significant.

The most important rules for individual clinical studies of nutrient effects suggested by Robert Heaney (see Box 1 below) are as follows (59).

Box 1 **Rules for individual clinical studies of nutrient effects.**

1. Basal nutrient status must be measured, used as an inclusion criterion for entry into study, and recorded in the report of the trial.
2. The intervention (i.e., change in nutrient exposure or intake) must be large enough to change nutrient status and must be quantified by suitable analyses.
3. The change in nutrient status produced in those enrolled in the trials must be measured and recorded in the report of the trial.
4. The hypothesis to be tested must be that a change in nutrient status (not just a change in diet) produces the sought-for effect.
5. Conutrient status must be optimized in order to ensure that the test nutrient is the only nutrition-related, limiting factor in the response.

OBJECTIVES

PRIMARY OBJECTIVE

The primary objective of this multicenter, placebo-controlled double-blind phase III RCT is to assess the effect of oral high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency.

SECONDARY/SAFETY OBJECTIVES

The secondary objectives of this trial are to evaluate if vitamin D3 affects morbidity and to ascertain safety of this intervention.

EXPLORATORY OBJECTIVES

Among the exploratory objectives of this trial, we aim to investigate if certain patient populations benefit more or less from vitamin.
This will be done by predefined subgroup analyses regarding kidney function and an admission diagnosis of sepsis as detailed later.

TRIAL DESIGN

The VITALIZED study is a pragmatic, multicenter, placebo-controlled double-blind randomized controlled phase III trial in adult critically ill patients which will be conducted in academic and non-academic centers. The sponsor is the Medical University of Graz, Austria, a large tertiary care facility with > 120 ICU beds with a catchment area of > 1.5 million inhabitants covering the southeast of Austria. Most trials sites will be in Austria, with additional sites in Germany, Belgium, and likely also in Switzerland and UK..

In total, 2400 subjects will be randomised in a 1:1 ratio to receive either of the two treatments:

- Vitamin D: oral/enteral pharmacological dose of cholecalciferol (vitamin D3)
    - total dose 900,000 IU
    - loading dose of 540,000 IU (dissolved in 37.5 ml of medium chain triglycerides - MCT) followed by 4000 IU daily (10 drops) for the entire active study period (90 days)
- Placebo: identical regime – loading dose of 37.5 ml MCT followed by 10 drops daily

This study uses a group sequential design, with one interim analysis when 50% of the planned enrolled patients in each arm (N=600 per arm) have completed their day 28 assessment by the independent data safety monitoring board. The enrollment of patients will continue while the interim analyses is performed.

EARLIEST START DATE

Q2 2017

RECRUITMENT PHASE

Anticipated 2-3 years based on estimated recruitment rates of 20-100 per center and year

END OF STUDY

The end of the study will be reached either at the interim analysis if the data safety monitoring board decides to stop the study prematurely. Otherwise, the study ends when 2400 patients have been included and completed visit 4 (day 90).

COENROLLMENT

Coenrollment may be allowed after careful review of the principal investigators and in case of unlikely biological interference between the VITDALIZE study and another study. This would be the case e.g. for a study assessing transfusion of older versus newer blood products, or different mechanical ventilation strategies. Likely coenrollment will only be executed in the United Kingdom.

OUTCOMES

PRIMARY OUTCOME

The primary outcome will be 28-day mortality (starting from day 0 when the study medication loading dose is given).

SECONDARY OUTCOMES

Efficacy outcomes

- 90-day mortality
- 1-year mortality
- ICU and hospital mortality
- Hospital and ICU length of stay (starting at day 0, ending at discharge from the trial site or day 90)
- SOFA day 5 (48 hours tolerance)
- Number of organ failures day 5 (0-6; > 2 SOFA points in each of the 6 categories)

- Laboratory: 25(OH)D and 1,25(OH)2D levels at day 5 (48 hours tolerance)
- Katz Activities of Daily Life (56) at day 90
- Self – reported infections requiring antibiotics until day 90
- Hospital and ICU readmission until day 90
- discharge disposition (home, rehabilitation, other hospital)

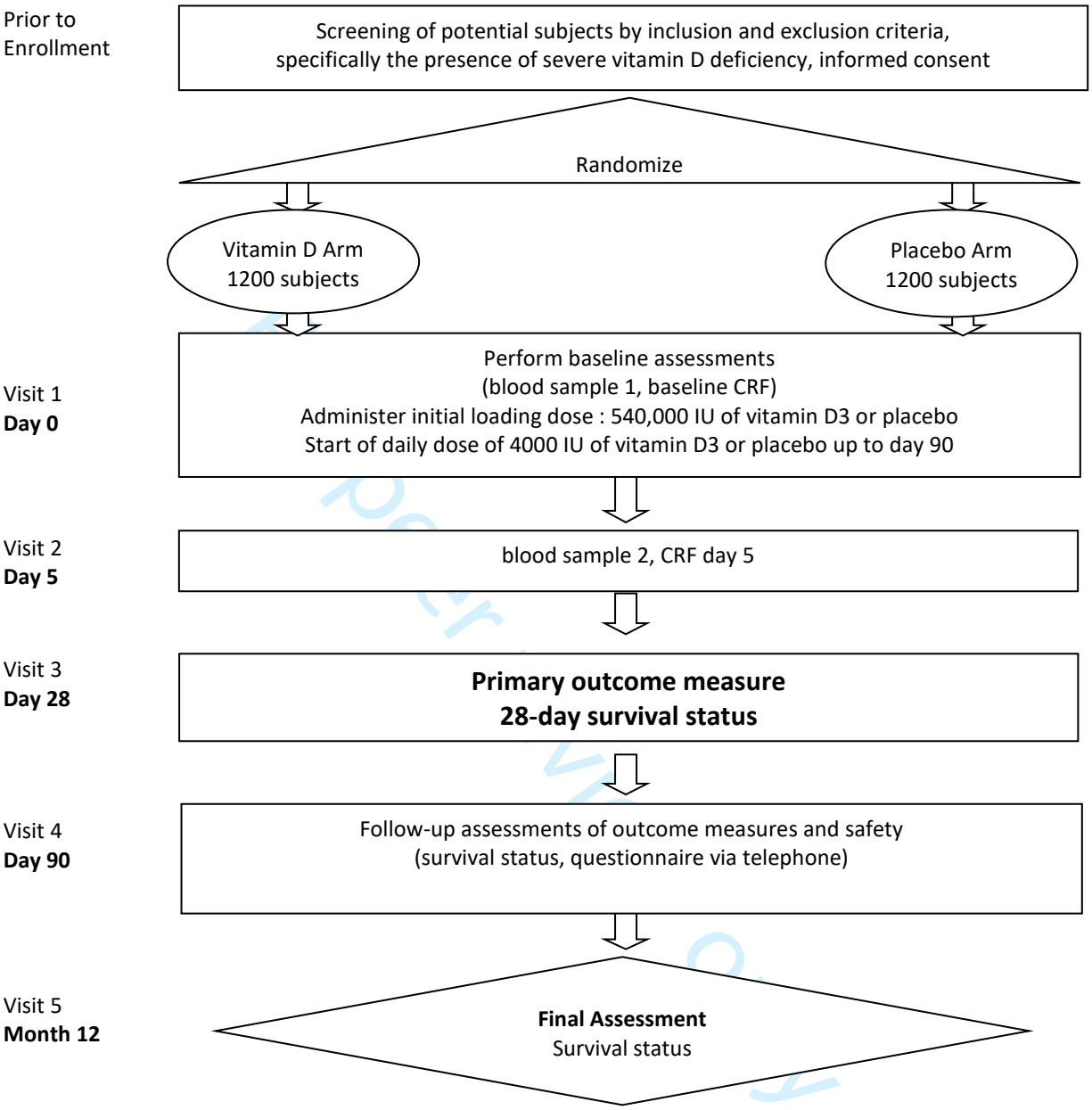
Safety outcomes

- Hypercalcemia at day 5 (48 hours tolerance)/during ICU stay
- Self-reported falls, fractures until day 90
- New episodes of kidney stones

PREDEFINED SUBGROUPS

- Kidney function (CKD 4 or lower vs. higher at inclusion)
- Sepsis (admission diagnosis) vs. non sepsis

FIGURE 1: Schematic Study Design



TRIAL POPULATION

The trial population consists of mixed adult critically ill patients anticipated to require > 48 hours of ICU care at the time of screening with documented vitamin D deficiency using local routine testing (25(OH)D ≤ 12 ng/ml (= 30 nmol/L) or undetectable) recruited in several countries in academic and non-academic hospitals.

INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

- Patients ≥18 years
- Anticipated ICU stay ≥ 48 hours
- Admission to ICU ≤ 72 hours before screening
- Severe vitamin D deficiency (≤ 12 ng/ml or undetectable) using local routine testing after ICU admission

EXCLUSION CRITERIA

- Severe gastrointestinal dysfunction/ unable to receive study medication
- DNR order/imminent death
- Hypercalcemia (> 2.65 mmol/l total calcium and/or > 1.35 mmol/l ionized calcium at screening)
- Known kidney stones, active tuberculosis or sarcoidosis (within the last 12 months)
- pregnancy/lactation (routine pregnancy test at ICU admission)
- other reasons (e.g. geographical reasons, diagnosed advanced dementia)
- hypersensitivity to drug or excipient

STUDY ENROLLMENT PROCEDURES/SUBJECT WITHDRAWAL CRITERIA

Local investigators will identify potential study participants within their ICU during routine care. A screening log documenting the reasons for exclusion from the trial will be kept within the electronic data management system Clincase.

Informed consent will be sought before study inclusion whenever possible. In case when this is not feasible at the time of study inclusion, surrogate or deferred informed consent will be acceptable. Country specific regulations apply as detailed in the ethical consideration section.

Withdrawal of a patient may be decided by the investigators, ie. in the case of severe hypercalcemia. In such cases, treatment may also be unblinded if the local investigator wishes to do so.

INTERVENTIONS

The intervention is a pharmacological dose of cholecalciferol (vitamin D3) versus placebo in an otherwise identical oily solution of medium chain triglycerides (MCT), either given by nasogastric or jejunal feeding tube or swallowed. We use the preparation that is commercially available in Austria (Oleovit, 12.5ml per bottle, 400 IU per drop, total dose of 180,000 IU per bottle).

In detail, the used intervention will be:

- Vitamin D: oral/enteral pharmacological dose of cholecalciferol (vitamin D3)
  - **total dose 900,000 IU**
  - loading dose of **540,000 IU** (dissolved in 37.5 ml of medium chain triglycerides - MCT) followed by **4000 IU daily** (10 drops) for the entire active study period (90 days)
- Placebo: identical regime – loading dose of 37.5 ml MCT followed by 10 drops daily

Rationale of dose

In our VITdAL-ICU study, we used the same loading dose, and this bolus dose achieved 25(OH)D levels > 30ng/ml on day 7 in 52% of the intervention group. After the first month, monthly maintenance doses of 90,000 IU were continued by 90% of the study population, and the patients or their caretakers were personally reminded every month by telephone. Although this is a simple approach, monthly personal reminders by telephone are not feasible in this multicenter setting, and a monthly regimen is likely easier forgotten than a daily dose. Furthermore, it may be more physiological and efficacious to use daily doses after the large loading dose used upfront during a time when some patients have severely impaired gastrointestinal function. In contrast to the VITdAL-ICU study, we therefore exchange the monthly maintenance dose of 90,000 (corresponding to 3000 IU daily) for a daily follow-up dose of 4000 IU cholecalciferol. This dose corresponds to the tolerable upper intake level as recommended by the Institute of Medicine in 2011 for adult patients including pregnant women (48). The total dose will therefore be 900,000 IU in 3 months as opposed to 990,000 in 6 months in the previous regimen. We aim to remind patients regularly of study medication intake by text messages (SMS) to their or their caregivers' cell phone if feasible.

Application of study medication

The route chosen is peroral because currently no high-dose monopreparation of vitamin D3 is available. Although recently an interventional trial has tested high-dose intramuscular vitamin D3 (58), intramuscular injections may not be feasible in many ICU patients (risk of bleeding

and infection). In case of severe vomiting within one hour after application of the loading dose, half the loading dose (1.5 bottles) will be repeated.
Daily doses of 10 drops (=4000 IU of cholecalciferol) will be added to enteral nutrition, if provided. It will be acceptable to give weekly doses of 2.5 ml (+25%)

Preparation and labelling of study medication
Unlabelled Oleovit (verum, cholecalciferol or vitamin D3) bottles, unfilled identical bottles and the placebo MCT solution will be provided by Fresenius Kabi.
The labelling, filling of placebo bottles and distribution of the study medication to the study centers will be performed at a certified pharmacy (Graz or Salzburg).

Concomitant interventions
Routine low-dose vitamin D supplementation (≤ 800 IU/day) is allowed during the study period and will be documented.

Adherence assessment
At visit 4 (Day 90), compliance will be assessed using the percentage of actually taken doses compared to the prescribed doses (self reported or reported by the caregivers).

Active vitamin D metabolites
Although in the future it will be interesting and necessary to assess the potential need for additional active vitamin D in critical illness, and specifically acute/chronic kidney failure, we choose not to do so in this trial because of simplicity and costs.

STEERING COMMITEE
The steering committee consists of the PI and the co-investigators.

VISIT PLAN / STUDY PROCEDURES

SCHEDULE OF EVALUATIONS

ASSESSMENT	Screening VISIT -1	Enrollment Baseline data, loading dose VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5
	Day -9 to Day 0	Day 0	Day 5	Day 28	Day 90	Month 12
Time window (days)			±2		±14	±30
25(OH)D	X					
inclusion/exclusion criteria	X	X				
signed informed consent if possible, otherwise deferred/surrogate	X	X				
randomisation		X				
demographics		X				
SAPS III		X				
TISS 28		X				
Charlson comorbidity index		X				
Stool sample (microbiome, subgroup) (optional, for Medical University Graz centers only)		X	X			
SAFETY EVALUATION						
Serum calcium	X	X	X	(X)		
Falls					X	
Fractures					X	
New episodes of nephrolithiasis					X	
Intervention						
Loading dose 540,000 IU		X				
Daily dose 4,000 IU			X	X	X	
Outcome variables						
Mortality			X	X	X	X
SOFA		X	X			
qSOFA		X				
Number of organ failures		X	X			
Infections requiring antibiotics					X	
Hospital and ICU readmission					X	

Discharge disposition					X	
Katz Activity of Daily Living		X			X	
25(OH)D (optional, for Medical University Graz centers only)		X	X			
1,25(OH)2D (optional, for Medical University Graz centers only)		X	X			

VISIT -1 (SCREENING)

25(OH)D screening is performed within clinical routine of the trial sites. Screening should be performed within the first 72 hours after ICU admission, and 25(OH)D routine testing should be available within 72 hours. After a patient has been identified to be eligible for the trial, the study medication should be given within 72 hours (max. 9 days).

If the major inclusion criterion of severe vitamin D deficiency is met, the other inclusion and exclusion criteria are evaluated by the local investigator/s at Visit 1. Informed consent will be ascertained whenever possible.

BASELINE ASSESSMENTS

- Age
- Sex
- ICU admission diagnosis
- ICU type
- Charlson comorbidity index
- SAPS III
- TISS 28
- 25(OH)D routine testing
- qSOFA
- Katz Activities of daily life

At baseline, data on demographic and clinical characteristics of the patients are obtained. At Centers of Medical University Graz it is planned to take a blood sample before the loading dose on day 0 and day 5 to ascertain biochemical response. The samples will be stored frozen at -70 C until batch analysis. In this subgroup (Medical University Graz), also stool samples will be collected for microbiome analysis on day 0 and day 5 . Stool samples are stored at -80°C

The Charlson comorbidity index, SAPS III and TISS 28 will be determined to be able to adjust for severity of illness and preexisting comorbidities.

RANDOMIZATION AND BLINDING

Patients will be randomly assigned to either placebo or vitamin D3 in a 1:1 ratio, using the web-based randomization service "Randomizer for Clinical Trials" developed at the Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz. Patients will be stratified according to trial site (ICU) and gender. An independent statistician will set up the study in the Randomizer.

The following method will be used to maintain the blind: the randomization list from the randomizer.at will be kept strictly confidential and no routine vitamin D testing is done after study inclusion. The independent statistician and unblinded pharmacist will keep treatment allocation information confidential until database lock.
In case of safety concerns (eg. severe hypercalcemia > 3.5 mmol/L), participants of the study may be unblinded by the local investigator at each participating site and/or the coordinating center. This will be done and documented with the Randomizer.

VISIT 1

Study medication
The day of the study medication loading dose is day 0. This is also the start for the calculation of the time dependent outcome data.

VISIT 2 (DAY 5 ±2)

At day 5, extensive clinical and laboratory data will be collected, including several measures of severeness of morbidity. It is likely that the majority of all patients will still be hospitalized. In the unlikely case a patient will be discharged before day 5 the data will be collected at discharge (day 3 or day 4).

VISIT 3 (DAY 28)

At visit 3, the primary endpoint (28-day mortality) will be assessed. For Austria, this information may be collected from Statistik Austria. Otherwise, this information will be obtained by the research team including nurses through the patient data management system and/or telephone.

VISIT 4 (Day 90±14d)

A follow up visit will be done by telephone 90 days after study inclusion by each study center: 1) family physician and/or 2) patient and/or 3) hospital). This visit will include important safety evaluations and secondary outcomes. This is also the end of the active intervention.

VISIT 5 (1 year ±1 month)

A final follow up visit (with results reported separately) will be done by telephone after 1 year. For Austria, Statistik Austria will be contacted first.

COMPLETION

The database lock will take place after the 90-day follow up of the last patient has been completed, all queries have been sufficiently well adressed and implemented in the database (applicable for the interim and the final analysis).

UNBLINDING FOR INTERIM AND FINAL ANALYSIS

For the formal planned interim analysis the DSMB statistician will get the unblinding list from the independent statistician responsible for the randomization procedure.
For the final analysis the unblinding list will be given to the study statisticians after database lock.

SAFETY ASSESSEMENTS

SAFETY MONITORING (SERUM CALCIUM, RENAL FUNCTION, FALLS AND FRACTURES)

Because of extremely high event rate in ICU patients and the well known risk profile of vitamin D3, safety monitoring will be restricted to known vitamin D-related adverse events.

Calcium and creatinine will be monitored regularly for routine purposes during ICU stay and recorded in the eCRF. Patients will be asked for new episodes of nephrolithiasis, falls and fractures at the 90-day-follow up telephone visit.

ADVERSE EVENT REPORTING

Only potential study drug–related adverse events (hypercalcemia, new episodes of nephrolithiasis, falls, and fractures) will be monitored and recorded up to 90 days.
Details will be specified in a separate document (SOP Reporting of SAE in intensive care – version June 2016. No separate reporting of rehospitalization or death will be performed because of the expectedly high event rates in the setting of critical illness.

STOPPING RULES FOR THE TRIAL OVERALL

A DSMB will monitor trial progress and safety. Should safety concerns evolve, the DSMB might recommend stopping the study at any time. There will be one planned interim analysis for efficacy. In case of overwhelming benefit the DSMB can recommend stopping the trial.

DATA SAFETY MONITORING BOARD (DSMB)

- ➔ Peter Suter, Prof. em., Critical Care, University Hospital Geneva
- ➔ Heike Bischoff-Ferrari, Prof., Department of Geriatrics, University of Zurich
- ➔ Martin Posch, Department of Statistics, Medical University of Vienna

STATISTICAL CONSIDERATIONS

The statistical analysis here presented will be detailed in a Statistical Analysis Plan (SAP). Should the SAP and this protocol differ, the methods in the SAP prevail. The SAP will be finalized before database lock for the planned interim analysis.

DEFINITION OF ANALYSIS SETS

Study participants who do not provide an informed consent after regaining consciousness and refuse to provide any more information are excluded from the study and will not be included in any statistical analysis.

INTENTION-TO-TREAT POPULATION

The primary analysis will be performed on the intention-to-treat population (ITT). The ITT will include all patients who receive at least the loading dose of the study medication.
All patients included here will be analysed according to the treatment assignment during randomisation.

PER PROTOCOL POPULATION

The per protocol population will include all patients who received the loading dose and have a compliance > 80%. Compliance is defined as self reported percentage of doses ingested until day 90. Other major protocol violations may also lead to exclusion of patients from the per protocol population. This will be discussed on an individual basis within the study team.

SAFETY POPULATION

The safety analyses will be based on the treated set, which is defined as all randomized patients who receive at least one dose of trial medication. All patients will be analysed according to the treatment they actually received.

DATA ANALYSIS

General aspects

All clinical and safety data collected in the study will be analysed with SAS v9.4 procedures in a Windows XP environment. Data will be presented as summary tables and, where appropriate, as plots. Continuous data will be described by means, standard deviations, medians and upper and lower quartiles unless otherwise stated. The number of observations and minimum and maximum values are also included. All descriptive summaries will be displayed to one more decimal place than actually measured. Categorical data will be summarized using frequencies and percentages.

Demographic and Baseline Characteristics

In a summary of demographic, baseline and diagnostic characteristics (age, weight, height, sex, SAPS III, Charlson comorbidity index, qSOFA - criteria, laboratory parameters,…) a comparison of the treatment groups will take place. To this end, appropriate descriptive and inferential statistics will be applied. Relevant medical history will be also displayed using summary statistics according to the two treatment groups.

Analysis of primary outcome

The primary outcome will be 28-day mortality defined as time from application of the study medication loading dose (day 0) to day 28 or death. Kaplan Meier estimates of survival curves in each treatment arm will be displayed. Group comparison will be made using a two-sided log rank test. Additionally, a hazard ratio with a 95% confidence interval will be computed from an univariate Cox-proportional hazards regression. Furthermore sensitivity analyses using multivariable Cox-proportional hazards regression will be performed adjusting for important clinical parameters. Details will be defined in the SAP.

Analysis of secondary outcomes

ICU, hospital mortality, 90-day mortality and 1-year mortality will be analysed as the primary outcome. ICU and hospital length of stay are defined as time between application of the study medication (day 0) and discharge at the primary ICU/hospital; readmissions and stays

at other hospitals will not be added, date and hour will be recorded, in case of a missing hour, the time 12am will be used.

At day 5 the SOFA Score is recorded. Organ failures (> 2 SOFA points in each of the 6 categories) will be reported in total and for each organ system separately. To compare the laboratory parameters 25(OH)D and 1,25(OH)2D levels ANCOVA will be used.

At day 90, Katz Activities of Daily Life Score, infections requiring antibiotics and hospital and ICU readmission will be assessed. The Self – reported infections requiring antibiotics and hospital and ICU readmission will be categorized as yes/no. Additionally, the number of infection episodes, number of antibiotics and -discharge disposition (home, rehabilitation, other hospital) will be presented. Comparison between groups will be performed using non-parametric tests and Chi-square tests.

Stool samples

In a subgroup, stool samples will be collected for microbiome analysis on day 0 and day 5. Stool samples are stored at -80°C. DNA extraction from stool samples will be performed by mechanical lysis with a MagnaLyser Instrument (Roche Diagnostics, Mannheim, Germany) and subsequent total bacterial genomic DNA isolation with the MagNA Pure LC DNA Isolation Kit III (bacteria, fungi) in a MagNA Pure LC 2.0 Instrument (Roche Diagnostics) according to the manufacturer's instructions. For amplification of bacterial 16S rRNA the template-specific sequence AGAGTTTGATCCTGGCTCAG and CTGCTGCCTYCCGTA, targeting the hypervariable region V1-V2 of the 16S rRNA gene, are used. PCR reactions for each sample are performed in triplicates. Subsequently the amplicons are purified according to standard procedures, quantified, pooled and sequenced with the MiSeq Reagent Kits v3 (600 cycles, Illumina, Eindhoven, Netherlands) according to manufacturer's instructions with 20% OhiX (Illumina). The generated FASTQ files are used for microbiota analysis.
Raw reads from Illumina MiSeq are pre-processed and filtered using MOTHUR v.1.31. Reads are de-noised using PyroNoise and chimera-filtered with UCHIME. Pyrosequencing errors are reduced with pre.cluster and non-bacterial sequences were also excluded. High quality reads are aligned to the SILVA database and taxonomy was assigned by MOTHUR's implementation of the ribosomal database project (RDP)-classifier followed by binning into phylotypes based on taxonomy. The shared file is then converted into a biom table and passed on to QIIME's (v.1.9.1) core_diversity.py command using non-phylogenetic parameters.

Analysis of safety outcomes

The safety outcomes, (hypercalcemia on day 5, new kidney stones, self-reported falls, and fractures until day 90) will be analysed as binary variables and compared with Chi-square tests.

Subgroup analysis

Predefined subgroup analyses will be performed for all primary and secondary outcomes based on the following group definitions as exploratory analysis:

- Kidney function (CKD 4 or lower vs. higher at inclusion)
- Sepsis (admission diagnosis) vs. non sepsis defined by the 2016 criteria (suspected infection/qSOFA on day 0 – respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less (57)

MISSING DATA HANDLING

All available data will be used in the analyses and data summaries. There will be no imputation of any missing data.

SAMPLE SIZE AND INTERIM ANALYSIS

Sample size considerations

The sample size is based on the primary endpoint 28-day mortality. In the VITdAL-ICU study (2014), 28-day mortality rates of 36% (37/102) in the placebo group and 20% (20/98) in the Vitamin D Group were observed (51). In this multicenter study we assume 28-day mortality rates of 25% in the placebo group versus 20% in the vitamin D group. We assume that baseline (placebo) mortality will be lower in this study because in contrast to the previous VITdAL-ICU study, academic and non-academic smaller sites with patients with a lower severity of illness will participate.

Using a fixed sample size design and a two-sided log-rank test for equality of survival curves with a two-sided alpha level of 5%, a sample size of n=1093 per group will be needed to achieve a power of 80% (total sample size of 2186). The **Table** below shows the sample sizes when the assumptions about 28-day mortality rates are varied between 25% - 35% for the Placebo group and between 20% - 30% for the vitamin D group as well as when the observed 28-day mortality rates from Amrein et al (2014) are assumed.

Placebo 28-day mortality	**25%**		**30%**		**Based on the VITdAL-ICU study**
Alpha level	0.05	0.05	0.05	0.05	0.05
Placebo proportion alive (π_P) at time t	**0.75**	**0.75**	0.70	0.70	**0.637**
VitD proportion alive (π_{VitD}) at time t	**0.80**	**0.81**	0.8	0.75	**0.796**
Hazard ratio $\ln(\pi_{VitD})/\ln(\pi_P)$	0.78	0.73	0.63	0.81	0.51
Power (%)	**80**	**90**	80	80	**80**
n per group	1093	1002	296	1245	129
Total number of events required	486	434	143	679	68
N Total	**2186**	**2004**	592	2490	**258**

Table: Different sample size scenarios using a two-sided log rank test

One interim analysis will be conducted when 50% of planned enrolled patients in each arm have completed their day 28 assessment (Visit 3) or prematurely discontinued the study. This interim analysis is intended to test for efficacy, i.e. the trial will be terminated after the interim analysis, if the main question can already be answered at this interim analysis.

Using a O'Brien-Fleming spending function (60) a total sample size of at least N=2194 (494 events) is required to achieve 80% power. With this sample size a hazard ratio of 0.78 (for survival rates of 0.8 in the Vitamin D group and 0.75 in the Placebo group corresponding to a 5% absolute 28-day mortality difference) can be detected, using a 2-sided log rank test with 0.05 alpha level assuming that the hazards are proportional. Accounting for a drop-out rate of approximately 10% yields a total sample size of N=2400 patients. A **power of 90%** would be achieved with a similar sample size if the treatment effect is larger (Hazard Ratio 0,73, or a 6% absolute 28-day mortality difference).

Planned interim analysis

One formal interim analysis by the Data Safety Monitoring Board (DSMB) will be performed at inclusion of 50% (N=1200) of patients having their day 28 assessment completed or discontinued the study. The interim analysis will take place approximately 12-18 months after start of the study. If the interim analysis shows a benefit for the vitamin D group, the DSMB may recommend early study termination.

The interim analysis will be performed only for the primary outcome 28 day mortality. The O' Brien-Fleming rule will be used to stop the trial early for efficacy. In detail, if the p-value of the log rank test is smaller than 0.003, then the trial can be stopped early by the DSMB.

Only the primary endpoint will be assessed for the interim analysis with an alpha level of 0.003. It is planned to test the secondary efficacy variables and the safety outcomes at an alpha level of 0.05. We will specify this and any deviations from the study protocol in the SAP.

DATA COLLECTION AND QUALITY ASSURANCE/DATA MANAGEMENT

DATA HANDLING

Every center will use a specific abbreviation (eg. Salzburg – SBG). Individual patients will be identified using a number (eg. First patient in Salzburg: SBG – 001).

DATA COLLECTION FORMS - ELECTRONIC CRF

Every center will have access to the electronic centralized case report form *Clincase* (Quadratek Data Solutions Ltd., Berlin, Germany). *Clincase* is a validated EDC (electronic data capture) and CDM (clinical data management) system for all types and phases of clinical trials and registries. It complies fully with FDA 21 CFR part 11 and EU GMP Annex 11 regulations. A backup paper version will be available to all centers.

QUALITY ASSURANCE AND MONITORING

Monitoring and audits shall be performed during the clinical trial for the purpose of quality assurance. Details will be specified in the Monitoring Manual.

ETHICAL CONSIDERATIONS AND COUNTRY-SPECIFIC INFORMED CONSENT PROCEDURE

The study will be performed according to national laws, the Declaration of Helsinki and current ICH-GCP guidelines and will be submitted to the Ethics Committees of each participating country.

INFORMED CONSENT PROCEDURE

One important aspect in critically ill patients is informed consent. The majority of patients will not be able to give informed consent at the time of study inclusion due to altered state of consciousness. Only a minority of patients will be able to give full informed consent during the acute setting.

Country specific regulations when immediate informed consent is not possible

Whenever possible, written informed consent will be obtained directly from the patient or from a legal surrogate. The majority of patients, however, will not be able to give informed consent due to acute illness (e.g. sepsis), intubation, mechanical ventilation and sedation. Only a minority of patients will be able to give full informed consent in the acute setting. Based on the VITdAL trial, we assume that >80% of patients will not be able to give informed consent at the time of randomization.
The following procedures will be applied:

- Patient with full consent or available legal surrogate → Immediate informed consent
- Patient not able to consent → country specific regulations as detailed below
- Patient recovers to full consent → retrospective informed consent

Patient information and informed consent will be handled according to the rules of "Good Clinical Practice" and the "Declaration of Helsinki". All eligible patients will undergo the consent process prior to randomization as described above using different forms.

Country specific regulations apply in case patient is not able to consent:

Austria:
Deferred/surrogate informed consent (IC)
The institutional ethical committee, similar to other states of the European Union, approves the use of "surrogate consent." Informed consent will be obtained at a later time point if the patient survives and regains mental capacity.

Germany:
"Konsiliararztverfahren": 1-2 independent physician/s assess/es supposed patient's will (if possible by contact of relatives).
Alternatively, a legal representative, e.g. relative or person in charge by the guardianship court, needs to be contacted after inclusion to provide informed consent.

Switzerland:
"Konsiliararztverfahren": one independent physician assesses inclusion/exclusion criteria. Additionally, a relative or person in charge by the guardianship court needs to be contacted to provide informed consent.

Other countries that may participate in this study are the Belgium, the Czech Republic, Denmark the United Kingdom and Canada. Country-specific regulations exist but are not discussed here.

REFERENCES

1. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab. 2009;94(1):26-34.
2. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29(6):726-76.
3. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.
4. Heaney RP. Assessing vitamin D status. Curr Opin Clin Nutr Metab Care. 2011;14(5):440-4.
5. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369(21):1991-2000.
6. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2011(7):CD007470.
7. Amrein K, Venkatesh B. Vitamin D and the critically ill patient. Curr Opin Clin Nutr Metab Care. 2012;15(2):188-93.
8. McNally JD, Menon K. Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance. Translational Pediatrics. 2013;2(3):99-111.
9. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. BMJ. 2014;349:g6330.
10. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2014;1:CD007470.
11. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, et al. Vitamin d with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin d trials. J Clin Endocrinol Metab. 2012;97(8):2670-81.
12. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2(1):76-89.
13. Bolland MJ, Grey A, Gamble GD, Reid IR. The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. Lancet Diabetes Endocrinol. 2014;2(4):307-20.
14. 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(2):R47.
15. Zhang YP, Wan YD, Sun TW, Kan QC, Wang LX. Association between vitamin D deficiency and mortality in critically ill adult patients: a meta-analysis of cohort studies. Crit Care. 2014;18(6):684.
16. Upala S, Sanguankeo A, Permpalung N. Significant association between vitamin D deficiency and sepsis: a systematic review and meta-analysis. BMC Anesthesiol. 2015;15:84.
17. de Haan K, Groeneveld AB, de Geus HR, Egal M, Struijs A. 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(6):660.
18. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41(2):580-637.
19. Danai PA, Sinha S, Moss M, Haber MJ, Martin GS. Seasonal variation in the epidemiology of sepsis. Crit Care Med. 2007;35(2):410-5.
20. Remmelts HH, van de Garde EM, Meijvis SC, Peelen EL, Damoiseaux JG, Grutters JC, et al. Addition of vitamin D status to prognostic scores improves the prediction of outcome in community-acquired pneumonia. Clin Infect Dis. 2012;55(11):1488-94.
21. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013;5(7):2502-21.
22. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Rheum Dis Clin North Am. 2012;38(1):125-39.
23. De Pascale G, Vallecoccia MS, Schiattarella A, Di Gravio V, Cutuli SL, Bello G, et al. Clinical and microbiological outcome in septic patients with extremely low 25-hydroxyvitamin D levels at initiation of critical care. Clin Microbiol Infect. 2015.
24. Rajakumar K, Greenspan SL, Thomas SB, Holick MF. SOLAR ultraviolet radiation and vitamin D: a historical perspective. Am J Public Health. 2007;97(10):1746-54.
25. Levine JA. Sick of sitting. Diabetologia. 2015;58(8):1751-8.
26. Perron RM, Lee P. Efficacy of high-dose vitamin D supplementation in the critically ill patients. Inflamm Allergy Drug Targets. 2013;12(4):273-81.
27. Zajic P, Amrein K. Vitamin D deficiency in the ICU - a systematic review. Minerva Endocrinol. 2014.

28. Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E, et al. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med. 2011;39(4):671-7.
29. Padhi R, Panda B, Jagati S, Patra SC. Vitamin D status in adult critically ill patients in Eastern India: An observational retrospective study. Lung India. 2014;31(3):212-6.
30. Moraes RB, Friedman G, Wawrzeniak IC, Marques LS, Nagel FM, Lisboa TC, et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics (Sao Paulo). 2015;70(5):326-32.
31. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, et al. Impact of anesthesia and surgery for congenital heart disease on the vitamin d status of infants and children: a prospective longitudinal study. Anesthesiology. 2013;119(1):71-80.
32. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360(18):1912-4.
33. Amrein K, Christopher KB, McNally JD. Understanding vitamin D deficiency in intensive care patients. Intensive Care Med. 2015;41(11):1961-4.
34. Lee P. Vitamin D metabolism and deficiency in critical illness. Best Pract Res Clin Endocrinol Metab. 2011;25(5):769-81.
35. 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(12):3170-9.
36. Thickett DR, Moromizato T, Litonjua AA, Amrein K, Quraishi SA, Lee-Sarwar KA, et al. Association between prehospital vitamin D status and incident acute respiratory failure in critically ill patients: a retrospective cohort study. BMJ Open Respir Res. 2015;2(1):e000074.
37. Dancer RC, Parekh D, Lax S, D'Souza V, Zheng S, Bassford CR, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax. 2015;70(7):617-24.
38. Quraishi SA, Litonjua AA, Moromizato T, Gibbons FK, Camargo CA, Jr., Giovannucci E, et al. Association between prehospital vitamin D status and hospital-acquired bloodstream infections. Am J Clin Nutr. 2013;98(4):952-9.
39. Quraishi SA, Litonjua AA, Moromizato T, Gibbons FK, Camargo CA, Jr., Giovannucci E, et al. Association between prehospital vitamin D status and hospital-acquired Clostridium difficile infections. JPEN J Parenter Enteral Nutr. 2015;39(1):47-55.
40. 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(1):97-107.
41. 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(1):63-72.
42. Madden K, Feldman HA, Smith EM, Gordon CM, Keisling SM, Sullivan RM, et al. Vitamin D Deficiency in Critically Ill Children. Pediatrics. 2012.
43. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, et al. The Association of Vitamin D Status With Pediatric Critical Illness. Pediatrics. 2012.
44. Amrein K, Quraishi SA, Litonjua AA, Gibbons FK, Pieber TR, Camargo CA, Jr., et al. Evidence for a U-shaped relationship between prehospital vitamin D status and mortality: a cohort study. J Clin Endocrinol Metab. 2014;99(4):1461-9.
45. Lee P, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? Intensive Care Med. 2009.
46. Vanek VW, Borum P, Buchman A, Fessler TA, Howard L, Jeejeebhoy K, et al. A.S.P.E.N. position paper: recommendations for changes in commercially available parenteral multivitamin and multi-trace element products. Nutr Clin Pract. 2012;27(4):440-91.
47. Krishnan A, 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(4):230-8.
48. 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(1):53-8.
49. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Dietary Reference Intakes for Calcium and Vitamin D. Institute of Medicine, 2011.
50. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.

51. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, 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(15):1520-30.
52. Higgins DM, Wischmeyer PE, Queensland KM, Sillau SH, Sufit AJ, Heyland DK. Relationship of vitamin D deficiency to clinical outcomes in critically ill patients. JPEN J Parenter Enteral Nutr. 2012;36(6):713-20.
53. von Restorff C, Bischoff-Ferrari HA, Theiler R. High-dose oral vitamin D3 supplementation in rheumatology patients with severe vitamin D3 deficiency. Bone. 2009;45(4):747-9.
54. Vieth R. Critique of the considerations for establishing the tolerable upper intake level for vitamin D: critical need for revision upwards. J Nutr. 2006;136(4):1117-22.
55. Landoni G, Comis M, Conte M, Finco G, Mucchetti M, Paternoster G, et al. Mortality in Multicenter Critical Care Trials: An Analysis of Interventions With a Significant Effect. Crit Care Med. 2015;43(8):1559-68.
56. Heyland DK, Stapleton RD, Mourtzakis M, Hough CL, Morris P, Deutz NE, et al. Combining nutrition and exercise to optimize survival and recovery from critical illness: Conceptual and methodological issues. Clin Nutr. 2015.
57. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-10.
58. 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(11):2313-20.
59. Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. Nutr Rev. 2014;72(1):48-54.
60. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979;35(3):549-56.

List of Abbreviations

25(OH)D	25-hydroxyvitamin D, native vitamin D
1,25(OH)2D	1,25-dihydroxyvitamin D, actie vitamin D
AE	Adverse Event
CDM	clinical data management
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EDC	Electronic data capture
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IOM	Institute of Medicine
JAMA	Journal of the American Medical Association
LKH	Landeskrankenhaus
MCT	Medium chain triglycerides
N	Number (typically refers to subjects)
NEJM	New England Journal of Medicine
PI	Principal Investigator
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
WHO	World Health Organization

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicentre, placebo-controlled double-blind phase III RCT (The VITDALIZE Study) Page 1
Trial registration	2a	ClinicalTrials.gov (identifier: NCT03188796) EudraCT-No.: 2016-002460-13 Page 2
	2b	All items from the World Health Organization Trial Registration Data Set yes
Protocol version	3	24th of January 2018, Version 1.3
Funding	4	BMBF fund (01KG1815), ESICM, NHS fund (17/147/33)

Roles and responsibilities	5a	Principle Investigator Austria Prof. Dr. med Karin Amrein Division for Endocrinology and Diabetology Medical University of Graz Auenbruggerplatz 15 8036 Graz, Austria Coordinating Investigator Germany Prof. Dr. med Patrick Meybohm Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy University Hospital Frankfurt Theodor-Stern-Kai 7 60590 Frankfurt am Main, Germany Coordinating Investigator Belgium Dr. med. Dr. rer. nat. Jean-Charles Preiser Department of Intensive Care Erasme University Hospital Route de Lennik 808 1070 Brussels, Belgium Coordinating Investigator UK Dr. rer. nat. Dhruv Parekh Institute of Inflammation and Ageing University of Birmingham Mindelsohn Way Birmingham, B15 2WB, UK Coordinating Investigator Switzerland Prof. Dr. med. Marco Maggiorini Institute for Intensive Care University Hospital Zurich Rämistrasse 100 8097 Zurich, Switzerland

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5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) Data Monitoring Safety Board (DMSB) Prof. Peter Suter Department of Internal Medicine Medical University of Geneva Rue Gabrielle-Perret-Gentil 4 1205 Geneva, Switzerland Prof. Heike Bischoff-Ferrari Department of Geriatrics University Hospital Zurich Rämistrasse 100 8091 Zurich, Switzerland Prof. Martin Posch Center for Medical Statistics, Informatics, and Intelligent Systems University of Vienna Spitalgasse 23 1090 Vienna, Austria Prof. Akos Heinemann Institute of Pharmacology Medical University Graz Universitätsplatz 4 8010 Graz, Austria
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention ➔ Page 3+4
	6b	Explanation for choice of comparators ➔ Page 4
Objectives	7	Specific objectives or hypotheses ➔ Page 4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) ➔ page 2+4

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Page 4; 14-18
Eligibility criteria	10	Inclusion and exclusion criteria for participants. Page 5+6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Page 4; 6+7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) Study protocol
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) Study protocol
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Study protocol
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Page 7
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Figure 1, Table 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Page 7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Page 7

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Study protocol
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Study protocol
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Study protocol
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Study protocol
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Study protocol
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Study protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Study protocol
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Study protocol

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page 8+9, Study protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Page 9
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Page 9

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Study protocol
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Page 9
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Study protocol
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Not applicable

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Study protocol V1.3 from 24th January 2018 (EudraCT 2016-002460-13), patient information, and informed consent were or will approved of all participating centres.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Not yet planned

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Study protocol
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Study protocol
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site NO
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Study protocol
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Page 2
	31b	Authorship eligibility guidelines and any intended use of professional writers YES, page 2
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code yes
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Not applicable
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a study protocol of a multicentre, placebo- controlled double-blind phase III RCT (The VITDALIZE Study)

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Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a study protocol of a multicentre, placebo- controlled double-blind phase III RCT (The VITDALIZE Study)

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ABSTRACT

Introduction: Observational studies have demonstrated an association between vitamin D deficiency and increased risk of morbidity and mortality in critically ill patients. Cohort studies and pilot trials have suggested promising beneficial effects of vitamin D replacement in the critical ill, at least in patients with severe vitamin D deficiency. As vitamin D is a simple, low-cost and safe intervention, it has potential to improve survival in critically ill patients.

Methods and analysis: In this randomised, placebo-controlled, double-blind, multicentre, international trial, 2,400 adult patients with severe vitamin D deficiency (25(OH)D ≤12ng/ml) will be randomised in a 1:1 ratio by randomizer.at to receive a loading dose of 540,000 I.U. cholecalciferol within 72 hours after ICU admission, followed by 4,000 I.U. daily for 90 days or identical placebo. Hypercalcemia may occur as a side effect, but is monitored by regular checks of the calcium level. The primary outcome is all-cause mortality at 28 days following randomisation. Secondary outcomes are: ICU, hospital, 90 day and 1 year mortality; hospital and ICU length of stay, change in organ dysfunction on day 5 as measured by Sequential Organ Function Assessment score (SOFA), number of organ failures; Hospital and ICU readmission until day 90; Discharge destination, self-reported infections requiring antibiotics until day 90 and health-related quality of life. Recruitment status is ongoing.

Ethics and dissemination: Ethical approval was obtained by the Ethics Committee of the University of Graz, and will be gained according to individual national processes. Upon completion, results will be published in a peer-reviewed scientific journal. The study findings will be presented at national and international meetings with abstracts on-line.

Trial registration: ClinicalTrials.gov (Identifier: NCT03188796),
EudraCT-No.: 2016-002460-13

Funding: The trial is funded by the NIHR HTA (17/147/33), by the BMBF (01KG1815), the ESICM, and by Fresenius Kabi.

STRENGTH and LIMITATIONS of the STUDY

- The VITDALIZE trial is a large, randomized, double-blind, placebo-controlled, international and multicentre trial.
- The trial will provide important information about efficacy of high dose vitamin D administration in critically ill patients with severe vitamin D deficiency and could be a simple, inexpensive intervention to improve patient outcomes and quality of life.
- Patients with severe vitamin D deficiency would most likely benefit from treatment.
- Challenges of the trial include the heterogeneous intensive care unit patient cohort and the high number of patients that takes the risk of recruitment failure.

- Due to the differing national processes in consenting and monitoring there may be some variance and each country will abide by their local ethics committee consenting procedures.

INTRODUCTION

Vitamin D has much broader effects on various metabolic activities than originally expected [1–3]. Many recent papers have demonstrated the pleiotropic effects of vitamin D. Vitamin D is a precursor of a steroid hormone with a specific nuclear receptor (vitamin D receptor), which regulates more than 1,000 genes and is also an important regulator of the immune system [4]. Besides regulating calcium homoeostasis, vitamin D has an influence on muscles, blood vessels, cell proliferation and differentiation, and autoimmune processes. Therefore, vitamin D deficiency causes skeletal and non-skeletal diseases and seems to predispose to a variety of respiratory, immune, infectious, neurologic and cardiovascular diseases.

25(OH)D is the major circulating vitamin D metabolite, and its measure best reflects an individual's vitamin D status. Thus, serum 25(OH)D is the generally accepted parameter for determining vitamin D status. Although the definition for vitamin D deficiency is still under debate, a cut-off of 25(OH)D ≤12ng/ml (=30 nmol/l) is uniformly considered to represent deficiency [5]. 25(OH)D levels below 12 ng/ml (or 30 nmol/l) hallmark a greatly increased risk for rickets, osteomalacia and decreased fractional calcium absorption. Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating of vitamin D deficiency is currently not routinely performed in the ICU.

To date, only a few studies have investigated high-dose vitamin D in critically ill patients with severe vitamin D deficiency [6–8]. The largest study to date, the VITdAL-ICU randomised, double-blind, placebo-controlled, single-centre trial included 492 critically ill patients with vitamin D deficiency (25(OH)D ≤ 20ng/ml) assigned to receive either vitamin D or placebo [6]. Vitamin D3 or placebo was given orally or via nasogastric tube at a dose of 540,000 I.U. followed by monthly maintenance doses of 90,000 I.U. for 5 months. The study provided no differences between vitamin D and placebo group concerning the primary outcome of hospital length of stay, hospital mortality or 6-month mortality. However, lower hospital mortality was observed in the severe vitamin D deficiency subgroup (25(OH)D ≤12 ng/ml, n=200 or 42% of the total population). As this was only a secondary endpoint in the predefined subgroup with severe vitamin D deficiency, this finding is hypothesis generating and prompted the current VITDALIZE study. We hypothesise that vitamin D replacement will improve patient`s outcome and quality of life in critically ill patients with severe vitamin D deficiency.

METHODS and ANALYSIS

Trial Design

The VITDALIZE trial is a multicentre, randomised, placebo-controlled, double-blind phase III trial targeting a sample size of 2,400 critically ill patients with severe vitamin D deficiency in more than 30 sites in Austria, Germany, Belgium, Switzerland and UK.

The aim of the trial is to determine if high-dose vitamin D3 improves clinical outcomes and is cost-effective in comparison to placebo in adult critically ill patients with severe vitamin D deficiency. An extended version of the protocol is added as a supplementary file (Protocol_24.01.2018-1.3).

Trial population

The trial population consists of mixed adult critically ill patients within 72 hours of ICU admission anticipated to require ≥ 48h of ICU care at the time of screening with documented vitamin D deficiency using local routine testing (25(OH)D ≤ 12 ng/ml (=30 nmol/l)) recruited in several countries in academic and non-academic hospitals.

A flowchart of study intervention is seen in Fig. 1 and a schedule of assessment and procedures in Table 1.

Assessment	**Screening (V0)**	**Enrolment, Baseline data (V1)**	**Clinical data (V2)**	**28-day mortality (V3)**	**90-day follow-up (V4)**	**1-year follow-up (V5)**
		Day 0	Day 5	Day 28	Day 90	Month 12
Inclusion/Exclusion criteria	X	X				
Informed consent*		X				
Demographics		X				
RANDOMISATION		X				
INTERVENTION						
Loading dose 540,000 I.U. vitamin D3		X				
Daily dose 4,000 I.U. vitamin D3			X	X	X	
OUTCOME VARIABLES						
Mortality			X	X	X	X
SOFA		X	X			
Infections requiring antibiotics					X	
Hospital and ICU readmission					X	
Katz activity of daily life		X			X	
SAFETY EVALUATION						
Serum calcium	X	X	X	(X)		
Falls/Fractures					X	
New episodes of nephrolithiasis					X	
Creatinine					X	

Table 1: Frequency and scope of study visits.

The day of the study medication loading dose is day 0 (V1). At day 5 (V2), extensive clinical data will be collected. The primary endpoint (28-day mortality) will be assessed at V3. A follow-up visit will be done by telephone 90 days after randomisation with patient or family physician. This visit will include important safety evaluations and secondary outcomes. A final follow-up visit will be done after 1 year.

Recruitment

The single-centre pilot study (VITdAL-ICU) was conducted at the Medical University of Graz, a large tertiary academic centre with 123 ICU beds. During a 2-year period, we were able to recruit n=492 critically ill adult patients with vitamin D deficiency (<20 ng/mL). Severe vitamin D deficiency was found in 42%, or 200 patients [6]. Current data from recruiting sites indicate that this is between 30-50%. We will need to recruit an average of approximately 2-3 patients

per month. Recruitment rates are based on previous ICU trials, the VITdAL-ICU study and an accepted screen failure rate of 2 out of 3 for severe vitamin D deficiency. Given the high prevalence of severe vitamin D deficiency and broad eligibility criteria we believe this is a conservative but realistic recruitment target.

The per-side recruitment will be monitored actively at nationally co-ordinated trial management group meetings. We will aim to identify barriers and solutions to help improve the recruitment. Advice from sites with a high level of recruitment will be disseminated via teleconference. Advice on how to maximise recruitment will be sought from our patient partners on a regular basis. If centres consistently fail to meet recruitment targets we will recruit additional sites whilst minimising the resources used in maintaining poorly recruiting sites or allow higher recruitment rates than planned in high-recruiting centres.

Randomisation and Blinding

Randomization and blinding will be performed using the secure and validated web-based randomization service "Randomizer for Clinical Trials" (Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Austria; available at: www.randomizer.at). Patients will be randomly assigned to either vitamin D or placebo in a 1:1 ratio and stratified according to centre and gender. To ensure equal group sizes a restricted randomization method will be used.

Allocation concealment will be ensured, as the service will not release the randomisation code until the patient has been recruited into the trial.

The following method will be used to maintain blinding: the blinding list will be kept strictly confidential and no routine vitamin D testing is done after study inclusion. An independent statistician and unblinded pharmacist will keep treatment allocation information confidential until database lock. Patient's group allocation will not be revealed until final statistical analysis. In case of safety concerns (e.g. severe hypercalcemia > 3.5 mmol/L), participants of the study may be unblinded by the local investigator at each participating site and/or the coordinating centre. This will be done and documented with the Randomizer.

Intervention

- **Vitamin D3 group** receiving a bolus of 540,000 I.U. vitamin D3, dissolved in medium chain triglycerides (MCT, 37.5 ml), at day 0 followed by 4,000 I.U. daily (10 drops) for 90 days, or
- **Placebo group** receiving 37.5 ml MCT solution at day 0 followed by 10 drops MCT for 90 days.

A concomitant routine low dose vitamin D intake of up to 800 I.U. daily is permitted, but very unlikely to have an effect in this 90-day time frame and population.
Randomisation is stratified by centre and gender.

Inclusion and Exclusion Criteria

Inclusion criteria

- Age ≥ 18 years
- Anticipated ICU stay ≥ 48 hours
- Admission to ICU ≤ 72 hours before screening
- Severe vitamin D deficiency (≤ 12 ng/ml (30nmol/L) or undetectable) using local routine testing after ICU admission

Exclusion criteria

- Severe gastrointestinal dysfunction /unable to receive study medication
- Patients with a do not resuscitate (DNR) order /imminent death
- Not expected to survive initial 48 hours of admission or treatment withdrawal imminent within 24 hours.
- Hypercalcemia (> 2.65 mmol/l total calcium and/ or >1.35 mmol/l ionized calcium at screening)
- Known kidney stones, active tuberculosis or sarcoidosis (within the last 12 months)
- Pregnancy/lactation
- Hypersensitivity to drug or excipient

Outcome Measures

The primary outcome of this trial will be all-cause mortality at day 28 after randomisation.
Secondary outcomes include 90-day and 1-year all-cause mortality, ICU and hospital mortality and length of stay, change in organ dysfunction on day 5 as measured by the SOFA score (Sequential Organ Function Assessment) and the number of organ failures (0-6; as defined by >2 SOFA points in each of the 6 categories). The six categories comprise the respiratory system (PaO_2/FiO_2), the nervous system (Glasgow coma scale), the cardiovascular system (mean arterial pressure OR administration of vasopressors required), the liver (Bilirubin), the coagulation (number of platelets) and the kidney (creatinine or urine output).
Further secondary endpoints are hospital and ICU readmission rate until day 90, discharge destination (home, rehabilitation, other hospital, self-reported infections requiring antibiotics until day 90 and the Katz activities of daily life, the most appropriate instrument to assess

functional status as a measurement of the patient's ability to perform basic activities independently, at day 90 will be collected using a questionnaire.

Mortality at day 28, day 90 and 1 year will be enquired by telephone, through hospital information system and national data linkage systems (where available). SOFA scores will be generated by collection of routinely collected clinical data.

Safety outcomes comprise hypercalcaemia at day 5, self-reported falls and fractures until day 90 and new episodes of kidney stones.

In the UK arm, additional secondary outcomes are Health-related quality of life (EQ-5D-5L, a standardized instrument for measuring generic health status) and a disability assessment (WHO-DAS 2.0 [WHO Disability Assessment Schedule 2.0], a generic assessment instrument for health and ability) at 90 days and 1 year. EQ-5D-5L and WHODAS 2.0 EQ-5D are designed for self-completion and as such captures information directly from the respondent. Further additional secondary endpoints in the UK arm are secondary health care utilisation in the first year (ICU and hospital length of stay, readmissions and utilisation of hospital and community care resources after hospital discharge one year after randomisation), from Hospital Episode Statistics, civil registry data held by NHS Digital and patient questionnaires and health economics analysis including cost effectiveness of screening for and treating vitamin D deficiency in critical illness and cost per quality-adjusted life year gained one year after randomisation and at end of life.

Monitoring

Patients will be monitored daily for unexpected serious adverse events until death or discharge and will be reported to the regulatory authorities, other participating centers and the manufacturer of the study medication. Following site-initiation the need for further site monitoring visits will be assessed on an individual and risk proportionate approach. Further site monitoring visits may be triggered by the following: poor quality data returns, repeated issues with randomisation (ineligible patients being entered), repeated issues with consenting, non-compliance with the protocol or good clinical practice (GCP), unusual data patterns or safety reporting issues. This may vary depending on national oversight processes.

The DSMB is responsible for safeguarding the interests of trial subjects, assessing the safety and efficacy of the interventions during the trial, and for monitoring the overall conduct of the clinical trial. The DSMB will assess the progress of the trial at regular intervals (~ 6 months) and will evaluate all safety data. In addition, the DSMB will evaluate the results of the interim efficacy analysis. It will recommend to the coordinating investigator and the sponsor whether to continue or stop the trial.

Sample size considerations

The sample size for this multinational study is based on the primary endpoint 28-day mortality. In the VITdAL-ICU study, 28-day mortality rates of 36% (37/102) in the placebo group and 20% (20/98) in the vitamin D Group were observed [6]. The VITDALIZE study has been designed to be sufficiently powered to detect a smaller, but clinically relevant absolute mortality difference of 5% with a power of 80% with an assumed baseline mortality rate of 25%. This corresponds to a clinically highly relevant relative risk reduction of 20%. Multicentre trials generally have a smaller treatment effect than monocentre studies [9]. We assume that this will also be the case for the VITDALIZE study. Furthermore, our assumed 5% absolute mortality difference is in line with a recent survey among clinical intensivists that the largest median treatment effect considered plausible by intensivists for current ongoing ICU multicentre trials is 3 to 5% [10].

Using a fixed sample size design and a two-sided log-rank test for equality of survival curves with a two sided alpha level of 5%, a sample size of N=1,093 per group will be needed to achieve a power of 80% (total sample size of 2,186).

Incorporating one interim analysis after inclusion of 50% of the patients a total sample size of at least N=2,194 (494 events) is required to achieve 80% power using a O'Brien-Fleming spending function [11]. Accounting for a drop-out rate of approximately 10% yields a total sample size of N=2,400 patients. For sample size calculation the software package nQuery 7.0 +nTerim 2.0 was used.

Statistical analysis

The primary analysis will be performed on the intention-to-treat population (ITT). The ITT will include all patients who receive at least the loading dose of the study medication. All patients will be analysed according to the treatment assignment during randomisation. The per protocol population will include all patients who received the loading dose and have a compliance > 80%. Compliance is defined as self-reported percentage of doses ingested until day 90.

The safety analyses will be based on the treated set, which is defined as all randomised patients who receive at least one dose of trial medication. All patients will be analysed according to the treatment they received.

Data analysis

All clinical and safety data collected in the study will be analysed with SAS v9.4. Data will be presented as summary tables and, where appropriate, as plots. Continuous data will be described by means, standard deviations, medians and upper and lower quartiles unless

otherwise stated. The number of observations and minimum and maximum values are also included.
Categorical data will be summarized using frequencies and percentages.

The primary outcome, 28-day all-cause mortality, will be displayed using Kaplan Meier estimates of survival curves in each treatment arm. Group comparison will be made using a stratified two-sided log rank test. The unstratified log-rank test will be performed as a sensitivity analysis. The Cox regression model, including treatment and stratification factor, will be used to estimate the hazard ration and its 95% CI. Details will be defined in a Statistical Analysis Plan.

ICU, hospital mortality, 90-day mortality and 1-year mortality will be analysed as secondary outcomes using Kaplan Meier estimates of survival curves. For the other secondary parameters, comparison between groups will be performed using appropriate parametric or non-parametric methods and Chi-square tests.

The safety outcomes, (hypercalcemia on day 5, new kidney stones, self-reported falls, and fractures until day 90) will be analysed as binary variables and compared with Chi-square tests.

Demographic and Baseline Characteristics
In a summary of demographic, baseline and diagnostic characteristics (age, weight, height, sex, SAPS III, Charlson comorbidity index) a comparison of the treatment groups will be performed. To this end, appropriate descriptive and inferential statistics will be applied. Relevant medical history will be also displayed using summary statistics according to the two treatment groups.

Subgroup analysis
Predefined subgroup analyses will be performed for all primary and secondary outcomes based on the following group definitions as exploratory analysis:
• Kidney function (CKD 4 or lower vs. higher at inclusion)
• Sepsis (admission diagnosis) vs. non-sepsis defined by the 2016 criteria (suspected infection/ qSOFA on day 0 – respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less)

Missing data handling

All available data will be used in the analyses and data summaries. There will be no imputation of any missing data.

Planned interim analysis

The trial uses a group sequential design with one interim analysis when 50% of the planned enrolled patients in each arm (N=600 per arm) have completed their day 28 assessment by the independent data safety monitoring board. The enrolment of patients will continue while the interim analysis is performed. This interim analysis is intended to test for efficacy, i.e. the trial may be terminated after the interim analysis, if the main question can already be answered at this interim analysis. If the interim analysis shows a benefit for the vitamin D group, the DSMB may recommend early study termination. The interim analysis will be performed only for the primary outcome 28-day mortality. The O' Brien-Fleming rule will be used to stop the trial early for efficacy. In detail, if the p-value of the log rank test is smaller than 0.003, then the trial can be stopped early by the DSMB.

DISCUSSION

Recent studies have demonstrated that low vitamin D levels are an independent risk factor for mortality in critically ill patients [12–17] reflecting the relevant role of vitamin D. Worldwide, the prevalence of vitamin D deficiency in intensive care patients ranges between 40-70%. Therapeutic interventions like surgery, fluid resuscitation, extracorporeal membrane oxygenation, cardiopulmonary bypass, dialysis and plasma exchange and hepatic, parathyroid and renal dysfunction may significantly reduce vitamin D levels [18]. Pleiotropic effects of vitamin D on the immune system, glucose metabolism, and calcium homeostasis are essential in critically ill patients. Vitamin D deficiency carries an additional risk due to mortality and morbidity to these patients. ICU patients often suffer from immunological dysfunction and changes in body composition (loss of muscle mass, increase in the adipose tissue). Every additional day staying on ICU increases the chance of becoming dependent on care with prolonged rehabilitation and recovery time. Interventions, such as vitamin D supplementation, may also have the potential to improve health related quality of life.

A recent Cochrane meta-analysis [19] with 50,623 adults who were healthy or were recruited among the general population, or diagnosed with a specific disease, showed that vitamin D3 supplementation was linked to significantly improved survival. An individual patient data analysis from 8 major vitamin D trials with > 70,000 participants showed a reduction of mortality by vitamin D by 9% [20]. Genetically low 25-Hydroxyvitamin D is associated with increased all-cause mortality [21]. An observational cohort study of 4,344 adults hospitalised between 1993 and 2011 demonstrated that in those patients with pre-hospital 25(OH)D

concentrations <20ng/ml, an improvement in vitamin D status during the year leading up to hospitalisation was independently associated with improved all-cause mortality and decreased hospital length of stay [22].

Recently, in a double-blind, randomised controlled study, it was demonstrated that the administration of high dose vitamin D (up to 500,000 IU) increased levels of anti-microbial molecules [23] that may have beneficial effects on critical illness and inflammatory outcomes. Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating of vitamin D deficiency is currently not routinely performed.

The VITDALIZE trial is a large randomized, multicentre international study designed to demonstrate the clinical benefit of vitamin D supplementation in critically ill patients with severe vitamin D deficiency. The primary outcome will be 28-day all-cause mortality. All-cause mortality represents a “hard” endpoint that is not prone to measurements bias. Most importantly, the European Medicines Agency recently recommended short-term (28-day) all-cause mortality as the most relevant primary efficacy endpoint in confirmatory clinical trials assessing the efficacy of drugs or medicinal products in patients with life-threatening acute illnesses, e.g. in critically ill patients with sepsis [24] or with acute respiratory distress syndrome [25]. Long-term effects will be reflected by the secondary endpoints 90 days and 1-year mortality and the secondary endpoint Katz Activity of Life will reflect the health related quality of life. The UK arm will also be assessing the cost-effectiveness and health economics with further health-related quality measures of the intervention.

Due to the differing national processes in consenting and monitoring there may be some variance and each country will abide by their local ethics committee consenting procedures.

The VIOLET (Vitamin D to improve outcomes by leveraging early treatment; ClinicalTrials.gov Identifier: NCT03096314) trial is another important and similar, yet substantially different RCT that has stopped recruitment in July 2018 but no results have been published at the time of writing. VIOLET included patients with vitamin D deficiency (point-of-care test, < 20ng/ml) in patients at risk for ARDS, but not necessarily ICU patients. The intervention consisted of a single bolus loading dose (540,000 I.U. vitamin D3), but no maintenance dose; and the primary endpoint is 90 day mortality. Together, these two large trials in acutely ill patients will greatly advance our knowledge in this field.

As vitamin D3 application is a simple, low-cost, safe and well-tolerated intervention, it has great potential to improve survival and quality of life in critically ill patients and could be implemented worldwide immediately.

ETHICS and DISSEMINATION

Study protocol (V1.3, EudraCT-No. 2016-002460-13), patient information, and informed consent were approved by the Ethics Committee of the University of Graz (EK 1289/2016), and will be submitted to each participating trial centre.

Each patient must give written informed consent to participate in the study.

Recruitment in Austria started in October 2017 and in Belgium in January 2019. The current study protocol (V1.3) was released in January 2018. This trial was registered at http://clinicaltrials.gov (identifier NCT03188796) in June 2017. So far, more than 300 patients have been randomised in > 15 active centres. The planned recruitment lasts for approximately another 36 months. The United Kingdom is funded by NIHR HTA and likely to start recruitment in 2020. Germany will be funded by the BMBF (Federal Ministry of Education and Research) and will start recruitment in 2020. In addition, ESICM and Fresenius Kabi support funding. The participation of Switzerland is planned, but will depend on funding possibilities.

Upon completion, results will be published in a peer-reviewed scientific journal.

List of abbreviations

MCT=Medium Chain Triglycerides; ICU=Intensive Care Unit; I.U.=International Units

Fig.1: Trial flow of intervention scheme

We will check eligibility and obtain informed consent* from patients or legally authorized representative/ health care proxy. After evaluation of exclusion criteria, patients will be randomised in the intervention or placebo group. Primary endpoint is 28-day all-cause mortality.

* When informed consent is not possible at time of screening, country-specific alternative strategies for obtaining informed consent are used (i.e. in Austria delayed informed consent, in Germany, consent of relatives, England and Wales consent of relatives or responsible clinician).

Abbreviations: ICU: Intensive Care Unit; IU: International Unit; MCT: Median Chain Triglycerides; SOFA: Sequential Organ Failure Assessment

REFERENCES

1. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab. 2009;94:26–34. doi:10.1210/jc.2008-1454.
2. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and

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3. Amrein K, Papinutti A, Mathew E, Vila G, Parekh D. Vitamin D and critical illness: What endocrinology can learn from intensive care and vice versa. Endocr Connect. 2018;7:R304-R315. doi:10.1530/EC-18-0184.
4. Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. J Autoimmun 2017. doi:10.1016/j.jaut.2017.07.007.
5. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. Public Health Nutr. 2011;14:938–9. doi:10.1017/S1368980011000565.
6. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, 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;312:1520–30. doi:10.1001/jama.2014.13204.
7. Leclair TR, Zakai N, Bunn JY, Gianni M, Heyland DK, Ardren SS, Stapleton RD. Vitamin D Supplementation in Mechanically Ventilated Patients in the Medical Intensive Care Unit. JPEN J Parenter Enteral Nutr 2019. doi:10.1002/jpen.1520.
8. Langlois PL, Szwec C, D'Aragon F, Heyland DK, Manzanares W. Vitamin D supplementation in the critically ill: A systematic review and meta-analysis. Clin Nutr 2017. doi:10.1016/j.clnu.2017.05.006.
9. Dechartres A, Boutron I, Trinquart L, Charles P, Ravaud P. Single-center trials show larger treatment effects than multicenter trials: evidence from a meta-epidemiologic study. Ann Intern Med. 2011;155:39–51. doi:10.7326/0003-4819-155-1-201107050-00006.
10. Ridgeon EE, Young PJ, Bellomo R, Mucchetti M, Lembo R, Landoni G. The Fragility Index in Multicenter Randomized Controlled Critical Care Trials. Crit Care Med. 2016;44:1278–84. doi:10.1097/CCM.0000000000001670.
11. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979;35:549–56.
12. 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. doi:10.1097/CCM.0b013e31822d74f3.
13. Moraes RB, Friedman G, Wawrzeniak IC, Marques LS, Nagel FM, Lisboa TC, Czepielewski MA. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics (Sao Paulo). 2015;70:326–32. doi:10.6061/clinics/2015(05)04.
14. 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. doi:10.1186/cc13790.
15. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360:1912–4. doi:10.1056/NEJMc0809996.
16. Venkatram S, Chilimuri S, Adrish M, Salako A, Patel M, Diaz-Fuentes G. Vitamin D deficiency is associated with mortality in the medical intensive care unit. Crit Care. 2011;15:R292. doi:10.1186/cc10585.
17. Arnson Y, Gringauz I, Itzhaky D, Amital H. Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. QJM. 2012;105:633–9. doi:10.1093/qjmed/hcs014.

18. McNally JD, Menon K. Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance. Transl Pediatr. 2013;2:99–111. doi:10.3978/j.issn.2224-4336.2013.07.03.
19. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2014:CD007470. doi:10.1002/14651858.CD007470.pub3.
20. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, et al. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin D trials. J Clin Endocrinol Metab. 2012;97:2670–81. doi:10.1210/jc.2011-3328.
21. Aspelund T, Grübler MR, Smith AV, Gudmundsson EF, Keppel M, Cotch MF, et al. Effect of Genetically Low 25-Hydroxyvitamin D on Mortality Risk: Mendelian Randomization Analysis in 3 Large European Cohorts. Nutrients 2019. doi:10.3390/nu11010074.
22. Amrein K, Litonjua AA, Moromizato T, Quraishi SA, Gibbons FK, Pieber TR, et al. Increases in pre-hospitalization serum 25(OH)D concentrations are associated with improved 30-day mortality after hospital admission: A cohort study. Clin Nutr. 2016;35:514–21. doi:10.1016/j.clnu.2015.03.020.
23. Han JE, Alvarez JA, Jones JL, Tangpricha V, Brown MA, Hao L, et al. Impact of high-dose vitamin D3 on plasma free 25-hydroxyvitamin D concentrations and antimicrobial peptides in critically ill mechanically ventilated adults. Nutrition. 2017;38:102–8. doi:10.1016/j.nut.2017.02.002.
24. Clinical investigation of medicinal products for the treatment of sepsis. Reference CPMP/EWP/4713/2003.
25. Clinical investigation of medicinal products in the treatment of patients with acute respiratory distress syndrome. Reference CPMP/EWP/504/97 Rev.1.

AUTHOR'S CONTRIBUTION

All authors have substantially contributed to the interpretation of current specific knowledge, which resulted in the conception and design of the present trial. KA, DP, SW, JCP, PE, PS, DT and PM are investigators of the present trial and participated in the acquisition of funding and contribute to the collection of data. AB and RR wrote the statistical methods. All authors have been involved in drafting the manuscript or revising it critically for important intellectual content, and approved the final manuscript.

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COMPETING INTEREST STATEMENT

This work will be supported by a fund by the BMBF (01KG1815), by the NIHR HTA (17/147/33), by Fresenius Kabi and the ESICM.

PATIENT and PUBLIC INVOLVEMENT STATEMENT

A patient and public group were involved in the UK arm set-up and continue to be actively involved in the trial co-ordination. Members have been involved in the development of the patient information and consent forms as well as UK protocol review and are co-applicants in the UK NIHR funding. They will play a major role in the dissemination of the study results.

WORD COUNT

3,395 words

ADDITIONAL MATERIAL

Study protocol, version 1.3 from 24th January 2018

ACKNOWLEDGEMENT

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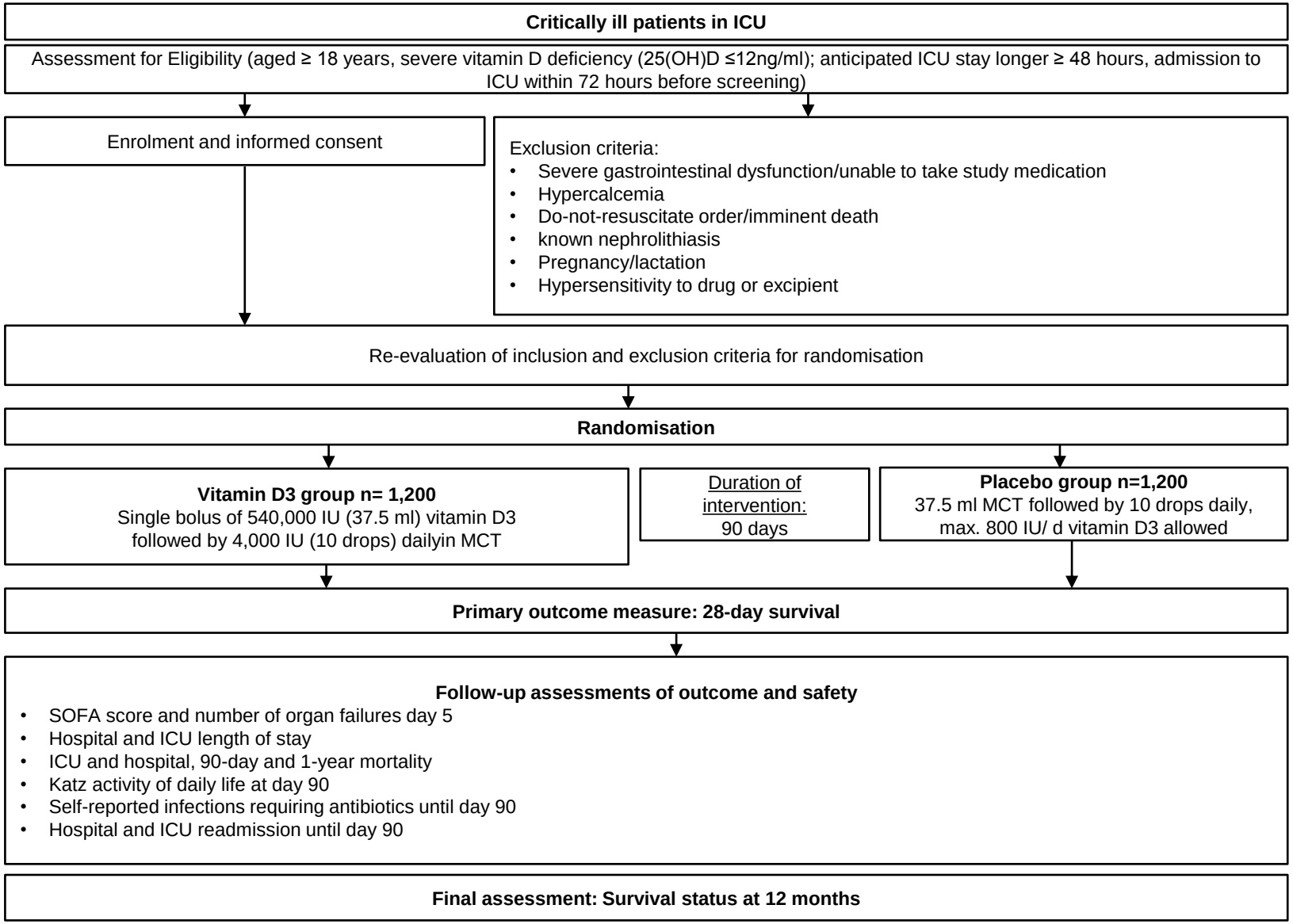
Critically ill patients in ICU
Assessment for Eligibility (aged ≥ 18 years, severe vitamin D deficiency (25(OH)D ≤12ng/ml); anticipated ICU stay longer ≥ 48 hours, admission to ICU within 72 hours before screening)
Enrolment and informed consent
Exclusion criteria:
• Severe gastrointestinal dysfunction/unable to take study medication
• Hypercalcemia
• Do-not-resuscitate order/imminent death
• known nephrolithiasis
• Pregnancy/lactation
• Hypersensitivity to drug or excipient
Re-evaluation of inclusion and exclusion criteria for randomisation
Randomisation
Vitamin D3 group n= 1,200
Single bolus of 540,000 IU (37.5 ml) vitamin D3 followed by 4,000 IU (10 drops) dailyin MCT
Duration of intervention: 90 days
Placebo group n=1,200
37.5 ml MCT followed by 10 drops daily, max. 800 IU/ d vitamin D3 allowed
Primary outcome measure: 28-day survival
Follow-up assessments of outcome and safety
• SOFA score and number of organ failures day 5
• Hospital and ICU length of stay
• ICU and hospital, 90-day and 1-year mortality
• Katz activity of daily life at day 90
• Self-reported infections requiring antibiotics until day 90
• Hospital and ICU readmission until day 90
Final assessment: Survival status at 12 months

PROTOCOL

The VITDALIZE Study

Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicenter, placebo-controlled double-blind phase III RCT

EudraCT number 2016-002460-13

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SYNOPSIS

STUDY NAME	The VITDALIZE Study: Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicenter, placebo-controlled double-blind phase III RCT
SPONSOR	Medical University of Graz Auenbruggerplatz 15 8036 Graz, Austria
STEERING COMMITTEE	Consists of the coordinating investigator and co-investigators
EARLIEST START DATE	Q2 2017
RECRUITMENT PHASE	2-3 years
FOLLOW UP	12 months
STUDY CENTER	Ca. 30 (ca. 15 non-academic)
PARTICIPATING COUNTRIES	Austria Germany Switzerland/UK/Belgium
RATIONALE & BACKGROUND	In the VITdAL-ICU trial using a large oral dose of vitamin D3 in 480 adult critically ill patients, there was no benefit **regarding the primary endpoint hospital length of stay. However, the predefined subgroup with severe vitamin D deficiency (25(OH)D ≤ 12ng/ml) had significantly lower 28-day mortality** (36.3% placebo vs. 20.4% vitamin D group, HR 0.52 (0.30-0.89), **number needed to treat = 6**). Therefore, high-dose vitamin D3 in a population of severely vitamin D deficient critically ill patients is a promising and inexpensive intervention that requires confirmatory multicenter studies. To date, **only 7 interventions (e.g. noninvasive ventilation or prone positioning) have ever demonstrated mortality benefit for ICU patients in multicenter trials.** In case of benefit, vitamin D treatment in critically ill patients could be immediately implemented worldwide.
TARGET NUMBER OF PATIENTS TO BE INCLUDED	Maximum 2400 patients (1200 per group) 1 interim analysis after 1200 patients

	The sample size is based on an anticipated 5% absolute mortality reduction assuming an overall 28-mortality of 25% in the placebo group
INTERVENTION	Cholecalciferol (vitamin D3) versus placebo: Day 0: One single bolus loading dose 540,000 IU of oral (or enteral) vitamin D3 followed by 4000 IU of vitamin D3 daily for the entire active study period (90 days) versus placebo (medium chain triglycerides, MCT) – total dose 900,000 IU vitamin D3
INCLUSION CRITERIA	- ≥18 years - Anticipated ICU stay ≥ 48 hours - Admission to ICU ≤ 72 hours before screening - Severe vitamin D deficiency (≤12 ng/ml or undetectable)
EXCLUSION CRITERIA	- Severe gastrointestinal dysfunction (> 400 ml residual volume)/unable to take study medication - DNR order/imminent death - hypercalcemia - known nephrolithiasis, active tuberculosis or sarcoidosis (within the last 12 months) - pregnancy/lactation - not deemed appropriate by study team/physician - hypersensitivity to drug or excipient
AIM OF THE TRIAL	To test if high-dose vitamin D3 is beneficial for the clinical outcome of adult critically ill adult patients with severe vitamin D deficiency
PRIMARY OUTCOME	**28-day mortality**
SECONDARY/SAFETY OUTCOMES	90-day mortality 1-year mortality ICU and hospital mortality Hospital and ICU length of stay SOFA Score at day 5 (48 hours tolerance) and number of organ failures (> 2 SOFA points in each of the 6 categories) Katz Activities of Daily Life (ADL) at day 90 Self - reported infections requiring antibiotics until day 90

	Hospital and ICU readmission until day 90 Discharge disposition Microbiome analysis day 0 and day 5 (optional, for Medical University Graz centers only) Laboratory:25OHD and 1,25OH2D at day 1 and 5 (optional, for Medical University Graz centers only) Safety outcomes: Hypercalcemia on day 5 (48 hours tolerance) Self reported falls, fractures until day 90 New episodes of kidney stones
FOLLOW UP PROCEDURE	by telephone, 3 months, by telephone, 12 months (mortality only)
RANDOMIZATION	randomizer.at stratification by ICU and gender
ETHICS COMMITTEE INFORMED CONSENT	Austria: Deferred/surrogate informed consent (IC) Germany: urgent approval of one legal substitute by court, surrogate IC by legal substitute, deferred IC Switzerland: Deferred IC, relatives and unrelated physician
ESTIMATED RECRUITMENT RATES	20-100 per center and year
DATA SAFETY MONITORING BOARD	Peter Suter, Geneva Heike Bischoff-Ferrari, Zurich Martin Posch, Vienna
TIMELINE	Submitted to ESICM Clinical Trials Group 12/2015 Submitted to Ethical Committee Graz Q3/2016 Submitted to Ministry of Health Q4/2016 Funding sources (KLIF) Q3 /2017 Recruitment Start Run-In-Phase Q4/2017 Rollout 2018-2021

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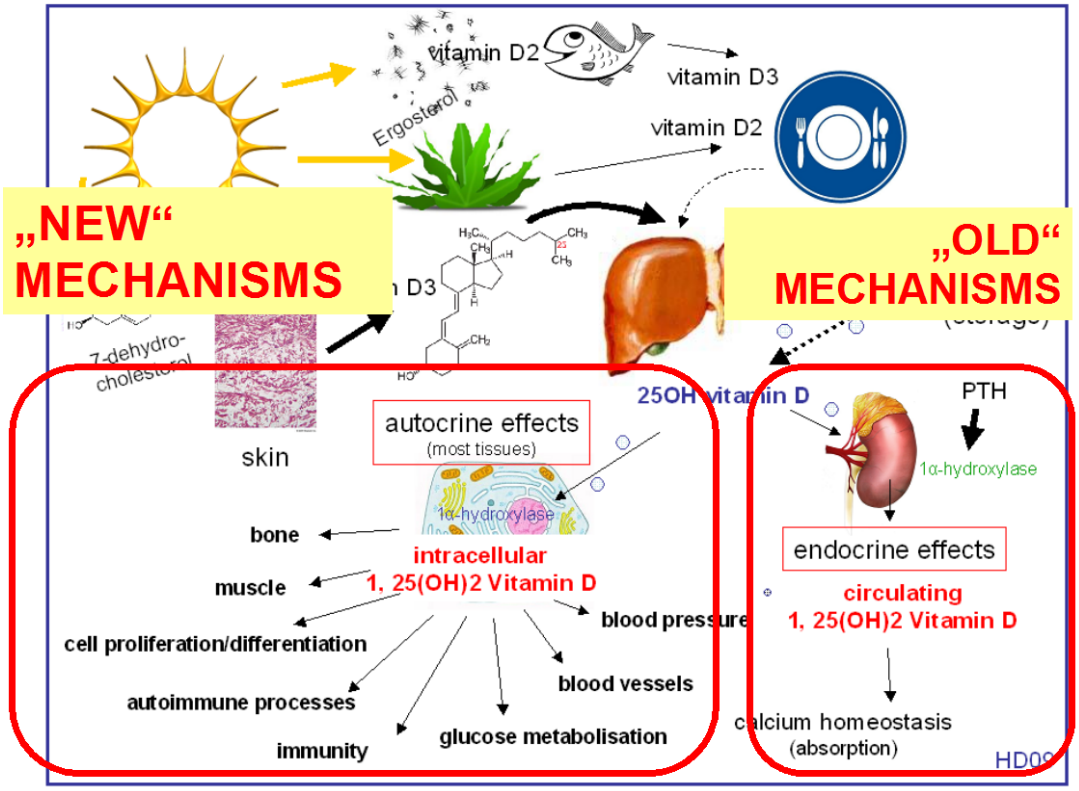
Title:

BACKGROUND

Traditionally, vitamin D has been thought to be primarily important to bone health, and its most severe form, rickets in children, seems to support this notion. However, in the last decade, vitamin D has seen an unprecedented revival, and currently several thousands of papers are published annually. This renewed interest was sparked by the finding that vitamin D has much broader, pleiotropic effects that extend well beyond the muskuloskeletal system (1) (overview in Figure 1, Dobnig H.). It is now estimated that vitamin D, which in reality is a precursor to a steroid hormone with a specific nuclear receptor (vitamin D receptor, VDR), regulates more than 200 genes and is also an important regulator of the immune system. Many of these findings have been studied in the vitamin D knockout mouse which has a variety of severe health problems and ages faster than wild-type mice (2). Vitamin D deficiency is more likely in patients with chronic diseases and limited mobility, but there is strong biological plausibility that supports a contributing role of vitamin D deficiency to poor outcomes.

The nuclear vitamin D receptor (VDR) is widely present in different cell types and organs that are relevant to critically ill patients via genomic and nongenomic pathways (2). The most important effects on target organs that are relevant during and after critical illness, namely muscle, heart, immune function, kidney and bone are summarized in the Figure.



Vitamin D needs are usually met with UV-B exposure from sunlight (or supplements), because

very few foods are rich in vitamin D (3). Endogenous vitamin D production is influenced by age, season, latitude and skin color. During the winter months, it is compromised at latitudes above 35°. During winter in the higher latitudes, sunlight has a longer tangential path to reach the earth's surface, resulting in the absorption and loss of the UV-B photons in the ozone stratosphere.

25(OH)D is the major circulating vitamin D metabolite, and its measure best reflects an individual's vitamin D status (4). Recently, there is a debate whether free vitamin D may be a better marker as there seem to be large interindividual differences (5), however the assay is currently not widely available.

Unfortunately, the enthusiasm is currently not supported by unequivocal clinical data, which is explainable by the small number of methodologically sound vitamin D intervention trials targeting patients with proven vitamin D deficiency at risk for high mortality and morbidity, similar to suboptimal clinical studies studying other nutrients (Heaney 2013). On the other hand, as opposed to many other interventions, vitamin D3 (cholecalciferol) has been shown to improve survival in a 2011 and a 2014 Cochrane metaanalysis (6).

The *link between vitamin D and critical illness is new (2009),* but intriguing, because

1) the majority of critically ill patients is vitamin D deficient,
2) standard care currently gives little or no vitamin D and
3) critically ill patients have a very high risk for mortality and morbidity.

VITAMIN D AND MORTALITY

At first glance, it seems absurd that one single substance should have such a profound effect. However, vitamin D deficiency causes skeletal and nonskeletal disorders in adults and children and seems to predispose to a variety of respiratory, immune, infectious, neurologic, cardiovascular and other diseases (3). There is also a strong association between a poor vitamin D status and excess morbidity and mortality in the general population, but also in critical illness, both in children and in adults (3, 7, 8).

Mendelian randomization studies

In 2014, the largest analysis (n>96,000) to date was published by Afzal et al. and evaluated overall mortality, cancer mortality and other mortalities (9). The odds ratio for a genetically determined 20 nmol/L lower plasma 25-hydroxyvitamin D concentration was 1.30 (1.05 to 1.61) for all cause mortality, with a corresponding observational multivariable adjusted odds ratio of 1.21 (1.11 to 1.31). Corresponding genetic and observational odds ratios were 0.77 (0.55 to 1.08) and 1.13 (1.03 to 1.24) for cardiovascular mortality, 1.43 (1.02 to 1.99) and 1.10

(1.02 to 1.19) for cancer mortality, and 1.44 (1.01 to 2.04) and 1.17 (1.06 to 1.29) for other mortality. The results were robust in sensitivity analyses. Each increase in DHCR7/CYP2R1 allele score was associated with a 1.9 nmol/L lower plasma 25-hydroxyvitamin D concentration and with increased all cause, cancer, and other mortality but not with cardiovascular mortality.

Cochrane metanalyses

Two consecutive Cochrane metaanalyses by Goran Bjelakovic with > 90,000 participants (2011 and 2014) showed that vitamin D3 (not other forms like vitamin D2 or active metabolites) supplementation was linked to significantly improved survival. Trial sequential analysis suggested a number needed to treat between 150 (2014) and 161 to prevent one additional death (2011; RR in both analyses 0.94, 95% CI 0.91 to 0.98). It has to be noted that most of the participants were elderly women > 70 years and only a small minority of the studies included patients with specific diseases (6, 10). In the 2014 analysis, vitamin D3 also statistically significantly decreased cancer mortality (RR 0.88; 95% CI 0.78 to 0.98).

Individual patient data (IPD) analysis

Lars Rejnmark published an IPD analysis using data fom 8 major vitamin D trials in 2012. The > 70,000 participants were mostly female (87%) and had a median age of 70 years. Mortality was reduced by vitamin D with calcium by 9% (HR 0.91, 95% CI 0.84-0.98), corresponding to a NNT of 151 (11).

Other, more critical reports

In a 2013 systematic review, Autier et al. came to the conclusion that “low 25(OH)D is a marker of ill health” but “an exception would be slight gains in survival after the restoration of vitamin D deficits” (12).

In a 2014 publication, Bolland et al. undertook a trial sequential meta-analysis of randomized controlled trials using vitamin D, with or without calcium, to investigate the effects of vitamin D supplementation on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, cancer, total fracture, hip fracture, and mortality predefining a risk reduction threshold of 5% for mortality and 15% for other endpoints in unselected community-dwelling individuals (13). They concluded that overall, vitamin D supplementation did not reduce these outcomes, although it reduced hip fracture in institutionalised individuals when co-administered with calcium and there was „uncertainty as to whether vitamin D with or without calcium reduces the risk of death“. Specifically, “vitamin D with or without calcium reduced the risk of death by 4% in traditional meta- analyses, but trial sequential analysis suggested that uncertainty remains in this finding“.

Vitamin D and hospital mortality

In our own observational dataset (n=655), all-cause hospital mortality was significantly higher in patients with vitamin D deficiency compared to patients with sufficient levels. In adjusted Cox regression analysis, compared to normal vitamin D levels, in vitamin deficiency the HR for hospital mortality was 1.63, 95% CI 0.93 to 2.86, and in vitamin D insufficiency 1.01 (95% CI: 0.52 to 1.96) (14).

A recent metaanalysis on hospital mortality in critically ill patients showed a significant association of vitamin D deficiency and increased hospital mortality (OR 1.76; 95% CI, 1.38 to 2.24; P <0.001) (15).

VITAMIN AND SEPSIS

Vitamin D deficiency is associated with an increased susceptibility of sepsis (16, 17). Sepsis is one the most common reasons for ICU admission and nosocomial infections frequently complicate and prolong ICU stay in other patients. The incidence of sepsis continues to rise and is the leading cause of death in critically ill patients, affecting millions of patients annually worldwide with a mortality rate of approximately 25% (18).

Sepsis incidence and mortality is also higher during the winter months when 25(OH)D concentrations are lower (19). In patients with pneumonia, vitamin D deficiency is associated with an increased risk of ICU admission and mortality (20).

The link between vitamin D status and sepsis is biologically plausible because vitamin D has important pleiotropic effects on the immune system (21, 22). Vitamin D metabolizing enzymes and vitamin D receptors are present in many cell types including various immune cells such as antigen-presenting-cells, T cells, B cells and monocytes. It regulates both the innate and the adaptive immune system and seems to increase antimicrobial peptides (cathelicidin, or LL-37 in its active form; and β-defensin), which are present in many epithelia of the human body, including the respiratory system and the urogenital tract.

A recent observational study in 107 Italian sepsis patients showed that 25(OH)D levels < 7ng/ml on admission were a major determinant of clinical outcome (23). Benefits of VD replacement therapy in this population should be elucidated.

VITAMIN D AND CRITICAL ILLNESS

Critical illness did not really exist until the accidental discovery of penicillin and the invention of the first simple ventilator, the iron lung (both between World War I and II). Even then, however, the use of both antibiotics and respirators was greatly limited by availability and the

absence of dedicated intensive care units. During the 1952 poliomyelitis outbreak in Denmark, > 2700, often young and previously healthy patients became ill within a few months, and > 300 needed respiratory support, which called for a different strategy. The anesthesiologist Dr. Ibsen then established positive pressure ventilation by tracheal intubation, and 200 medical students were deployed to manually ventilate the patients. At this time, the first positive pressure volume controlled ventilators were developped and eventually replaced the medical students. Subsequently, mortality declined from 90% to around 25%. Patients were treated in three special 35-bed units, the precursors to modern intensive care units (ICU). The first Critical Care Residency was established at the University of Pittsburgh in 1962 and the Society of Critical Care Medicine was founded in 1970.

Widespread vitamin D deficiency is probably also of relatively recent origin, after the industrialization substantially changed our lifestyle with air pollution, urbanization and decreased time spent outdoors (24). The invention of the modern sitting office workplace in the 1940s likely contributes to this development, as not few individuals spend up to 15 hours per day sitting and the time spent outdoors is very limited (25).

Prevalence of vitamin D deficiency in the ICU

The prevalence of vitamin D deficiency in intensive care ranges typically between 40 and 70 % (8, 26-30). There are many reasons to be or become deficient in the ICU. Moreover, therapeutic interventions like surgery, fluid resuscitation, extracorporeal membrane oxygenation, cardiopulmonary bypass, dialysis and plasma exchange may significantly reduce vitamin D levels (31). Hepatic, parathyroid and renal dysfunction also places ICU patients at risk for disruption of vitamin D metabolism.

In a 2009 letter to the New England Journal of Medicine, Paul Lee was the first to publish that vitamin D deficiency in the ICU is a common problem based on data from 42 patients referred for endocrinological evaluation (32). This finding has been replicated and extended (33, 34). There are now many observational studies that consistently show an association between a poor vitamin D status and poor clinical outcomes (8, 26, 27, 34).

It is now clear that in critical illness

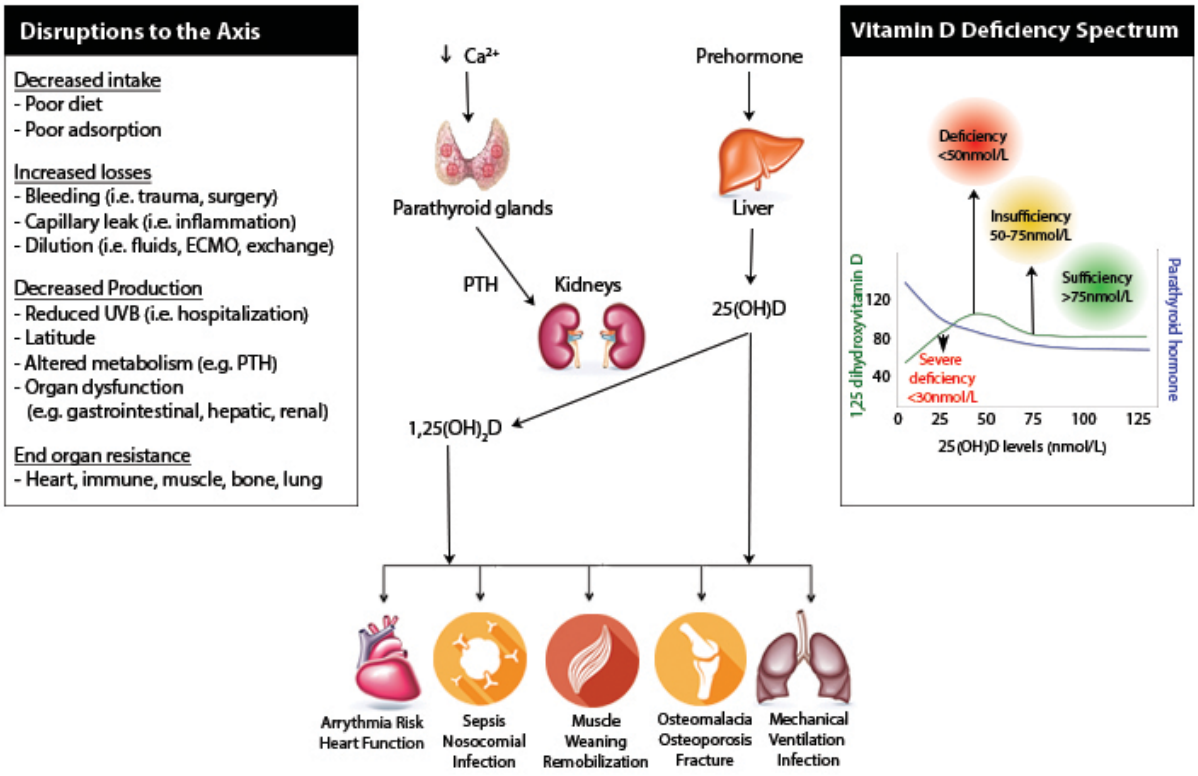
- the prevalence of vitamin D deficiency in ICU is very high
- vitamin D deficiency is associated with
    - excess morbidity
        - acute kidney injury (35)
        - acute respiratory failure, duration of mechanical ventilation and ARDS (36, 37)
        - sepsis, infections, positive blood cultures (28, 38-40)
    - excess mortality (adults and children) (14, 28, 41-44)

In summary, it may well be the case that vitamin D deficiency is indeed an "invisible accomplice to morbidity and mortality", as proposed earlier by Paul Lee (45).

Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating vitamin D deficiency is currently not routinely performed.

Vitamin D metabolism is disrupted in many ICU patients (overview in Figure below; reproduced from (33)). Additionally, many – especially medical – ICU patients enter the ICU in a deficient state because of preexisting poor lifestyle including malnutrition and preexisting disease.

The current ESPEN Guideline for parenteral nutrition in intensive care recommends All PN prescriptions should include a daily dose of multivitamins and of trace elements. (Grade C). The available parenteral (and enteral) multivitamin preparations contain very low doses, typically 200-250 IU per vial/table. Unfortunately, no intravenous high-dose vitamin D preparation is commercially available, although this is clearly needed, especially in patients with gastrointestinal malfunction, a common problem in critical illness (46).



Diagnosis of vitamin D deficiency in the ICU

In the general population, serum 25(OH)D is the generally accepted marker for determination of vitamin D status, however several issues regarding timing of the blood sample, assay specifics and the choice of metabolite are discussed (4, 47). Thresholds and terminology vary, but the most widely used definitions are as follows (to convert nmol/L to ng/ml, divide by 2.5) (3, 48):

	IOM Report 2011	**Holick M., NEJM 2007**
Vitamin D deficiency	< 12ng/ml	< 20ng/ml
Vitamin D sufficiency	> 20ng/ml	> 30ng/ml
Intoxication/potentially harmful	> 50ng/ml	>149ng/ml

These thresholds are based on biochemical indicators of axis stress and values below which the incidence for calcium malabsorption, secondary hyperparathyroidism and skeletal manifestation rises (49).

30 nmol/l (=12ng/ml) has been a cut point below which individuals were considered to have a risk of "vitamin D deficiency" and was utilized in many publications as well as in the full version of the "Institute of Medicine's 2011 Dietary Reference Intakes" report. 25(OH)D levels below 12 ng/ml (or 30 nmol/l) hallmarked an increased risk for rickets, osteomalacia and decreased fractional calcium absorption. The following summary and figure is given on p. 368: *"The congruence of the data links serum 25(OH)D levels below 30 nmol/L with the following outcomes: increased risk of rickets, impaired fractional calcium absorption, and decreased bone mineral content (BMC) in children and adolescents; increased risk of osteomalacia and impaired fetal skeletal outcomes; impaired fractional calcium absorption and an increased risk of osteomalacia in young and middle-aged adults; and impaired fractional calcium absorption and fracture risk in older adults".* Furthermore, on page 370, the following statement is given: *"The lower end of the requirement range is consistent with 30 nmol/L, and deficiency symptoms may appear at levels less than 30 nmol/L depending upon a range of factors".*

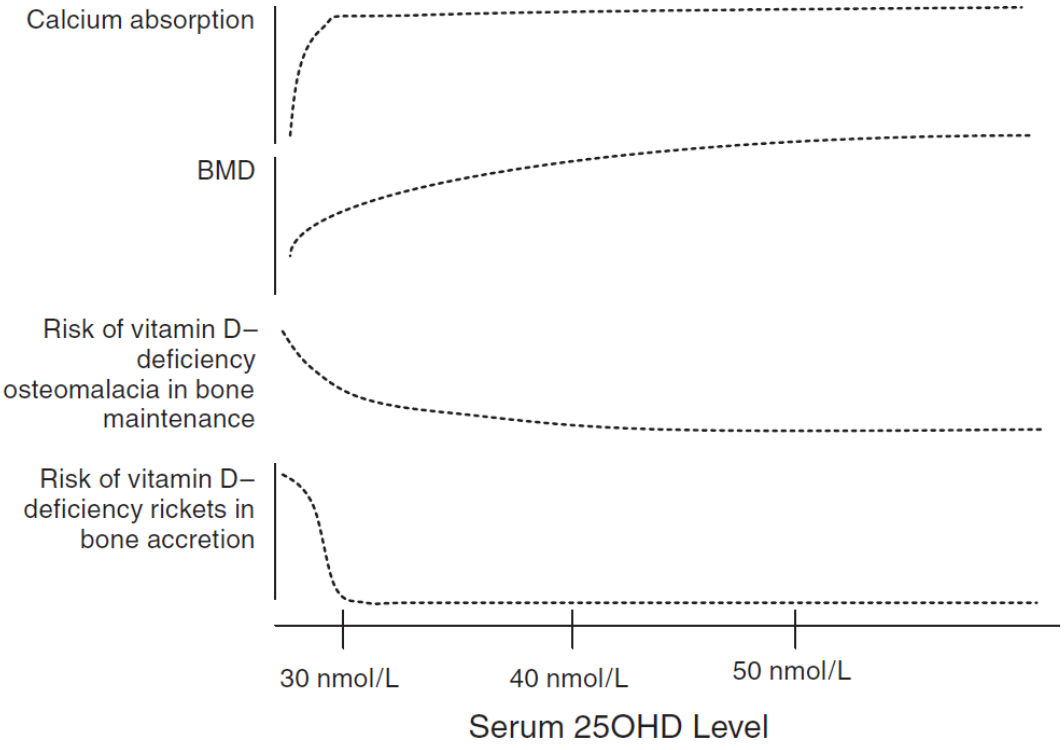


FIGURE 5-1 Conceptualization of integrated bone health outcomes and vitamin D exposure.

Current routine for vitamin D testing and supplementation in the ICU

In the general population, it is recommended that all healthy children and adults meet a daily minimum of vitamin D - the Institute of Medicine (IOM) recommends 400 to 800 IU of native vitamin D (48). The Endocrine Society recommends 1500 to 2000 IU for adult patients "at risk" for vitamin D deficiency (50).

In critical illness however, no standard of care has been established. Typical enteral and/or parenteral nutrition formulas supply ~ 200 -400 (<1000 IU) per day. In healthy individuals, such doses can prevent or improve vitamin D deficiency, but this requires months.

Rapid improvement of vitamin D status - justification of a high-dose vitamin D3 bolus

In hospitalized patients on low-dose vitamin D provided by nutrition formulas, 25(OH)D levels either remain constant or fall over time (51, 52). In critical illness, this approach does not substantially affect vitamin D levels in a meaningful time frame. Therefore, a bolus dose is an attractive option to rapidly improve vitamin D levels. The relatively long half-life of 25(OH)D following oral cholecalciferol supplementation allows for large loading doses of vitamin D3 which also have been shown to rapidly and safely normalize 25(OH)D levels in elderly patients

with vitamin D deficiency (53). Smaller doses of vitamin D3 are similarly or more effective but require a much longer period until a plateau of 25(OH)D levels can be reached.

In addition to the recommended daily allowance (RDA), an age specific daily upper tolerable intake levels of vitamin D has been suggested (1000 to 10,000 IU) (49, 50). The IOM uses a safety factor of 2.5, thus considering a permanent daily dose of 4000 IU of vitamin D to be safe (48).
The VITdAL-ICU study, the only phase III study evaluating rapid correction of vitamin D deficiency, provided a single enteral 540 000 IU loading dose of cholecalciferol to 237 critically ill adults. This dose increased 25(OH)D from 13 to 35 ng/ml until day 3 and did not provide evidence of safety concerns (51). Only very few patients achieved a 25(OH)D level of > 50ng/ml at any time point.

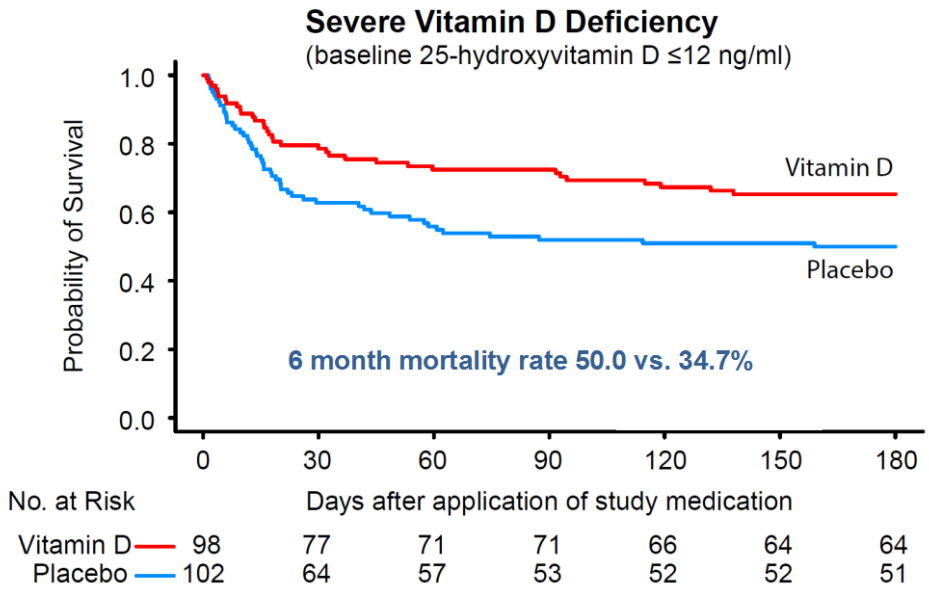
Supraphysiologic levels are not necessary, and there is evidence for a U-shape between 25(OH)D levels and outcomes (44). Vieth concluded in a review on the issue of "vitamin D toxicity" that only prolonged intakes of vitamin D at doses of >10,000 to 40,000 IU/day and 25(OH)D levels >200 ng/ml were shown to be associated with hypercalcemia (54). The daily follow-up dose of 4000 IU used in this study corresponds to the upper limit recommendation by the IOM (48).

Vitamin D intervention trials in the ICU

A very limited number of intervention trials, most including less than 30 patients, have been published. The only phase III study, our VITdAL-ICU study recruited from 2010 to 2012 and (n=475) did not find a difference in the primary endpoint „length of hospital stay" between placebo and high-dose vitamin D3. However, there was a non-significant absolute risk reduction in all-cause hospital mortality in the total population. The difference was larger (17.5%) and significant in the predefined subgroup of patients with severe vitamin D deficiency at baseline, see Kaplan Meier curve below (n=200, 28.6 vs 46.1%, p=0.01, 0.56 (0.35-0.90)), corresponding to a number needed to treat of 6. (51)
As this was only a secondary endpoint in the predefined subgroup with severe vitamin D deficiency, this finding is hypothesis generating and requires further study, leading to this application.
In our study, we were unable to identify a mechanism by which this benefit was achieved. Interestingly, looking at the causes of death, the vitamin D group seemed to benefit in every category (see below).

Table 2. Length of Stay and Mortality Outcomes for the Total and Subgroup Populations

	Total Study Population (N = 475)			Prespecified Subgroup Population					
				Severe Vitamin D Deficiency[a] (n = 200)			Less-Severe Vitamin D Deficiency[b] (n = 275)		
	Placebo (n = 238)	Vitamin D_3 (n = 237)	*P* Value	Placebo (n = 102)	Vitamin D_3 (n = 98)	*P* Value	Placebo (n = 136)	Vitamin D_3 (n = 139)	*P* Value
Length of stay, median (range)									
Hospital, d[c]	19.3 (0.1-154.1)	20.1 (0.2-181)	.98	19.0 (1.0-154.1)	20.1 (0.2-181)	.40	20.5 (0.1-113.9)	20.1 (0.2-133)	.47
ICU, d	10.7 (0.1-154.1)	9.6 (0.2-181)	.38	9.1 (0.8-154.1)	9.7 (0.2-181)	.98	12.3 (0.1-113.9)	9.0 (0.2-127)	.26
Mortality, No. (%)									
ICU	63 (26.5)	54 (22.8)		34 (33.3)	23 (23.5)		29 (21.3)	31 (22.3)	
HR (95% CI)	0.97 (0.67-1.39)		.86	0.70 (0.41-1.19)		.18[d]	1.32 (0.79-2.20)		.28[d]
28-d	68 (28.6)	52 (21.9)		37 (36.3)	20 (20.4)		31 (22.8)	32 (23.0)	
HR (95% CI)	0.76 (0.53-1.09)		.14	0.52 (0.30-0.89)		.02[d]	1.06 (0.64-1.73)		.83[d]
Hospital	84 (35.3)	67 (28.3)		47 (46.1)	28 (28.6)		37 (27.2)	39 (28.1)	
HR (95% CI)	0.81 (0.58-1.11)		.18	0.56 (0.35-0.90)		.01[d]	1.12 (0.72-1.77)		.61[d]
6-mo	102 (42.9)	83 (35.0)		51 (50.0)	34 (34.7)		51 (37.5)	49 (35.3)	
HR (95% CI)	0.78 (0.58-1.04)		.09	0.60 (0.39-0.93)		.02[d]	0.95 (0.64-1.41)		.81[d]
Causes of death, No. (%)									
Sepsis	30 (29.4)	26 (31.3)		16 (31.4)	12 (25.3)		14 (27.5)	14 (28.6)	
Cardiovascular	30 (29.4)	24 (28.9)	.99	13 (25.5)	9 (26.5)	.95	17 (33.3)	15 (30.6)	.98
Neurologic	19 (18.6)	14 (16.9)		8 (15.7)	4 (11.8)		11 (21.6)	10 (20.4)	
Other	23 (22.5)	19 (22.9)		14 (27.5)	9 (26.5)		9 (17.6)	10 (20.4)	



To date, only 7 interventions have ever demonstrated a mortality benefit for ICU patients in multicenter trials (see Table below, e.g. noninvasive ventilation or prone positioning), often only in relatively small subgroups such as resuscitated patients or in ARDS (55). NNTs ranged between 3 and 11 with the exception of tranexamic acid in trauma patients. In case of similar benefit, vitamin D treatment in critically ill patients could be immediately implemented worldwide.

Treatment	Centers	Patients	p	Absolute Risk Reduction	Relative Risk Reduction	Number Need to Treat to Save One Life	Follow-Up	Stopped at Interim Analysis	Blinding
Albumin in hepatorenal syndrome (3)	7	126	0.01	0.191	0.668	5	Hospital discharge*; 90 d*	No	Yes
Daily interruption of sedatives (17)	4	336	0.01	0.134	0.232	7	28 d; 1 yr*	No	No
Mild hypothermia (4)	9	275	0.02	0.142	0.258	7	Hospital discharge, 6 mo*	No	No
Noninvasive ventilation (5)	5	85	0.02	0.193	0.675	5	Hospital discharge*	No	No
Noninvasive ventilation (18)	3	50	0.009	0.2	0.714	5	60 d*	No	No
Noninvasive ventilation (19)	14	236	0.05	0.101	0.498	10	Hospital discharge*	No	No
Noninvasive ventilation (20)	3	105	0.028	0.213	0.548	5	ICU discharge*; 90 d*	No	No
Noninvasive ventilation (21)	2	162	0.025	0.142	0.871	8	ICU discharge*; hospital discharge; 90 d*	No	No
Noninvasive ventilation (22)	11	90	0.015	0.12	0.828	7	Hospital discharge*	No	No
Noninvasive ventilation (23)	3	106	0.0244	0.197	0.64	5	ICU discharge; hospital discharge; 90 d*	No	No
Noninvasive ventilation (24)	3	82	0.014	0.122	0.836	8	Hospital discharge*; 6 mo*; 1 yr*	No	No
Prone position (6)	27	474	< 0.001	0.168	0.512	6	28 d; 90 d*	No	No
Protective ventilation (7)	2	53	< 0.001	0.329	0.465	3	ICU discharge*; hospital discharge; 28 d*	Yes	No
Protective ventilation (8)	10	861	0.007	0.088	0.222	11	Hospital discharge*	Yes	No
Protective ventilation (25)	8	103	0.017	0.238	0.441	4	ICU discharge*; hospital discharge*; 28 d*	Yes	No
Tranexamic acid (26)	247	20,211	0.0035	0.015	0.094	68	Hospital discharge*	No	Yes

*Significant.

The most important rules for individual clinical studies of nutrient effects suggested by Robert Heaney (see Box 1 below) are as follows (59).

Box 1 **Rules for individual clinical studies of nutrient effects.**

1. Basal nutrient status must be measured, used as an inclusion criterion for entry into study, and recorded in the report of the trial.
2. The intervention (i.e., change in nutrient exposure or intake) must be large enough to change nutrient status and must be quantified by suitable analyses.
3. The change in nutrient status produced in those enrolled in the trials must be measured and recorded in the report of the trial.
4. The hypothesis to be tested must be that a change in nutrient status (not just a change in diet) produces the sought-for effect.
5. Conutrient status must be optimized in order to ensure that the test nutrient is the only nutrition-related, limiting factor in the response.

OBJECTIVES

PRIMARY OBJECTIVE

The primary objective of this multicenter, placebo-controlled double-blind phase III RCT is to assess the effect of oral high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency.

SECONDARY/SAFETY OBJECTIVES

The secondary objectives of this trial are to evaluate if vitamin D3 affects morbidity and to ascertain safety of this intervention.

EXPLORATORY OBJECTIVES

Among the exploratory objectives of this trial, we aim to investigate if certain patient populations benefit more or less from vitamin.
This will be done by predefined subgroup analyses regarding kidney function and an admission diagnosis of sepsis as detailed later.

TRIAL DESIGN

The VITALIZED study is a pragmatic, multicenter, placebo-controlled double-blind randomized controlled phase III trial in adult critically ill patients which will be conducted in academic and non-academic centers. The sponsor is the Medical University of Graz, Austria, a large tertiary care facility with > 120 ICU beds with a catchment area of > 1.5 million inhabitants covering the southeast of Austria. Most trials sites will be in Austria, with additional sites in Germany, Belgium, and likely also in Switzerland and UK..

In total, 2400 subjects will be randomised in a 1:1 ratio to receive either of the two treatments:

- Vitamin D: oral/enteral pharmacological dose of cholecalciferol (vitamin D3)
    - total dose 900,000 IU
    - loading dose of 540,000 IU (dissolved in 37.5 ml of medium chain triglycerides - MCT) followed by 4000 IU daily (10 drops) for the entire active study period (90 days)
- Placebo: identical regime – loading dose of 37.5 ml MCT followed by 10 drops daily

This study uses a group sequential design, with one interim analysis when 50% of the planned enrolled patients in each arm (N=600 per arm) have completed their day 28 assessment by the independent data safety monitoring board. The enrollment of patients will continue while the interim analyses is performed.

EARLIEST START DATE

Q2 2017

RECRUITMENT PHASE

Anticipated 2-3 years based on estimated recruitment rates of 20-100 per center and year

END OF STUDY

The end of the study will be reached either at the interim analysis if the data safety monitoring board decides to stop the study prematurely. Otherwise, the study ends when 2400 patients have been included and completed visit 4 (day 90).

COENROLLMENT

Coenrollment may be allowed after careful review of the principal investigators and in case of unlikely biological interference between the VITDALIZE study and another study. This would be the case e.g. for a study assessing transfusion of older versus newer blood products, or different mechanical ventilation strategies. Likely coenrollment will only be executed in the United Kingdom.

OUTCOMES

PRIMARY OUTCOME

The primary outcome will be 28-day mortality (starting from day 0 when the study medication loading dose is given).

SECONDARY OUTCOMES

Efficacy outcomes

- 90-day mortality
- 1-year mortality
- ICU and hospital mortality
- Hospital and ICU length of stay (starting at day 0, ending at discharge from the trial site or day 90)
- SOFA day 5 (48 hours tolerance)
- Number of organ failures day 5 (0-6; > 2 SOFA points in each of the 6 categories)

- Laboratory: 25(OH)D and 1,25(OH)2D levels at day 5 (48 hours tolerance)
- Katz Activities of Daily Life (56) at day 90
- Self – reported infections requiring antibiotics until day 90
- Hospital and ICU readmission until day 90
- discharge disposition (home, rehabilitation, other hospital)

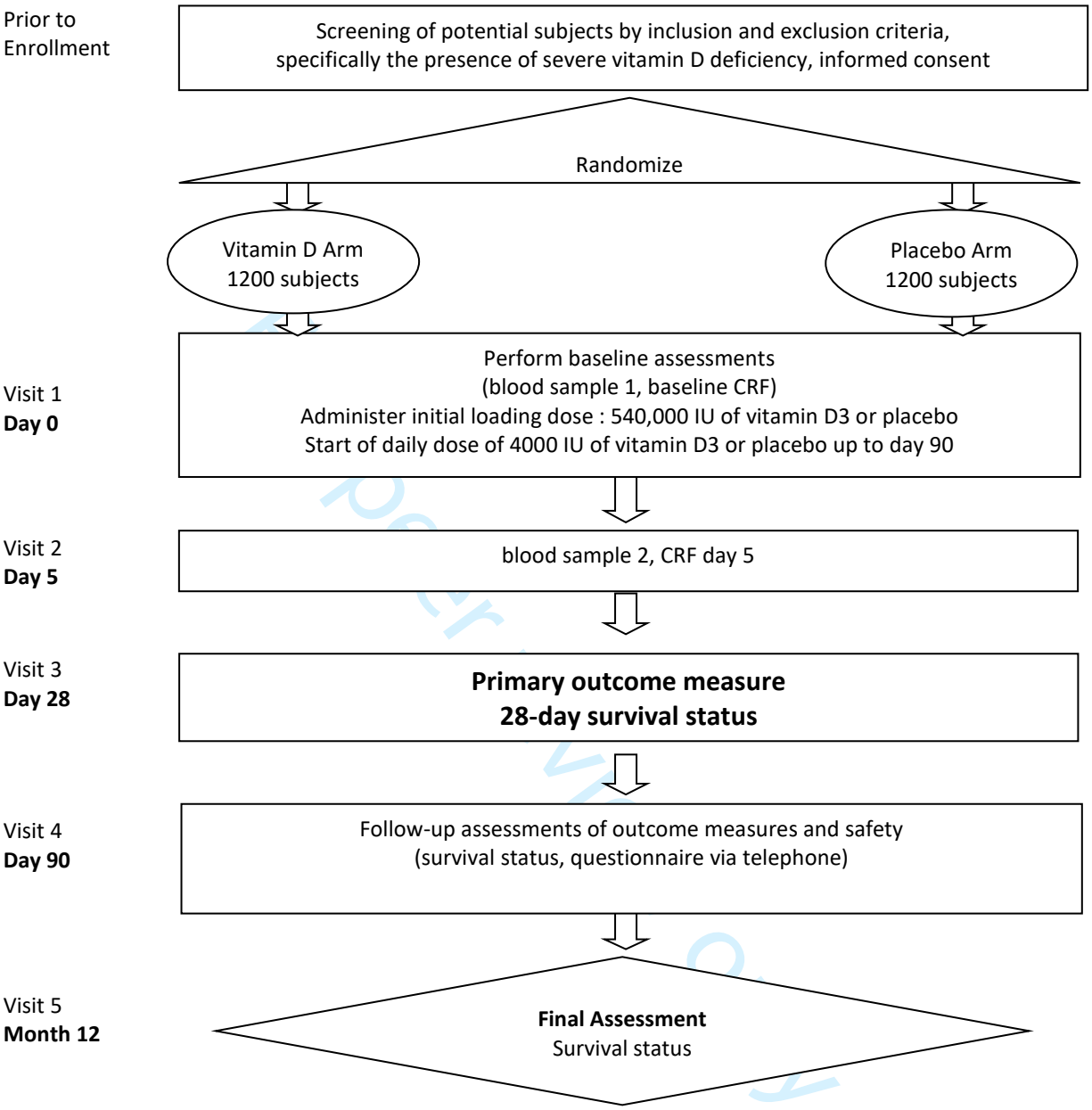
Safety outcomes

- Hypercalcemia at day 5 (48 hours tolerance)/during ICU stay
- Self-reported falls, fractures until day 90
- New episodes of kidney stones

PREDEFINED SUBGROUPS

- Kidney function (CKD 4 or lower vs. higher at inclusion)
- Sepsis (admission diagnosis) vs. non sepsis

FIGURE 1: Schematic Study Design



TRIAL POPULATION

The trial population consists of mixed adult critically ill patients anticipated to require > 48 hours of ICU care at the time of screening with documented vitamin D deficiency using local routine testing (25(OH)D ≤ 12 ng/ml (= 30 nmol/L) or undetectable) recruited in several countries in academic and non-academic hospitals.

INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

- Patients ≥18 years
- Anticipated ICU stay ≥ 48 hours
- Admission to ICU ≤ 72 hours before screening
- Severe vitamin D deficiency (≤ 12 ng/ml or undetectable) using local routine testing after ICU admission

EXCLUSION CRITERIA

- Severe gastrointestinal dysfunction/ unable to receive study medication
- DNR order/imminent death
- Hypercalcemia (> 2.65 mmol/l total calcium and/or > 1.35 mmol/l ionized calcium at screening)
- Known kidney stones, active tuberculosis or sarcoidosis (within the last 12 months)
- pregnancy/lactation (routine pregnancy test at ICU admission)
- other reasons (e.g. geographical reasons, diagnosed advanced dementia)
- hypersensitivity to drug or excipient

STUDY ENROLLMENT PROCEDURES/SUBJECT WITHDRAWAL CRITERIA

Local investigators will identify potential study participants within their ICU during routine care. A screening log documenting the reasons for exclusion from the trial will be kept within the electronic data management system Clincase.

Informed consent will be sought before study inclusion whenever possible. In case when this is not feasible at the time of study inclusion, surrogate or deferred informed consent will be acceptable. Country specific regulations apply as detailed in the ethical consideration section.

Withdrawal of a patient may be decided by the investigators, ie. in the case of severe hypercalcemia. In such cases, treatment may also be unblinded if the local investigator wishes to do so.

INTERVENTIONS

The intervention is a pharmacological dose of cholecalciferol (vitamin D3) versus placebo in an otherwise identical oily solution of medium chain triglycerides (MCT), either given by nasogastric or jejunal feeding tube or swallowed. We use the preparation that is commercially available in Austria (Oleovit, 12.5ml per bottle, 400 IU per drop, total dose of 180,000 IU per bottle).

In detail, the used intervention will be:

- Vitamin D: oral/enteral pharmacological dose of cholecalciferol (vitamin D3)
  - **total dose 900,000 IU**
  - loading dose of **540,000 IU** (dissolved in 37.5 ml of medium chain triglycerides - MCT) followed by **4000 IU daily** (10 drops) for the entire active study period (90 days)
- Placebo: identical regime – loading dose of 37.5 ml MCT followed by 10 drops daily

Rationale of dose

In our VITdAL-ICU study, we used the same loading dose, and this bolus dose achieved 25(OH)D levels > 30ng/ml on day 7 in 52% of the intervention group. After the first month, monthly maintenance doses of 90,000 IU were continued by 90% of the study population, and the patients or their caretakers were personally reminded every month by telephone. Although this is a simple approach, monthly personal reminders by telephone are not feasible in this multicenter setting, and a monthly regimen is likely easier forgotten than a daily dose. Furthermore, it may be more physiological and efficacious to use daily doses after the large loading dose used upfront during a time when some patients have severely impaired gastrointestinal function. In contrast to the VITdAL-ICU study, we therefore exchange the monthly maintenance dose of 90,000 (corresponding to 3000 IU daily) for a daily follow-up dose of 4000 IU cholecalciferol. This dose corresponds to the tolerable upper intake level as recommended by the Institute of Medicine in 2011 for adult patients including pregnant women (48). The total dose will therefore be 900,000 IU in 3 months as opposed to 990,000 in 6 months in the previous regimen. We aim to remind patients regularly of study medication intake by text messages (SMS) to their or their caregivers' cell phone if feasible.

Application of study medication

The route chosen is peroral because currently no high-dose monopreparation of vitamin D3 is available. Although recently an interventional trial has tested high-dose intramuscular vitamin D3 (58), intramuscular injections may not be feasible in many ICU patients (risk of bleeding

and infection). In case of severe vomiting within one hour after application of the loading dose, half the loading dose (1.5 bottles) will be repeated.
Daily doses of 10 drops (=4000 IU of cholecalciferol) will be added to enteral nutrition, if provided. It will be acceptable to give weekly doses of 2.5 ml (+25%)

Preparation and labelling of study medication
Unlabelled Oleovit (verum, cholecalciferol or vitamin D3) bottles, unfilled identical bottles and the placebo MCT solution will be provided by Fresenius Kabi.
The labelling, filling of placebo bottles and distribution of the study medication to the study centers will be performed at a certified pharmacy (Graz or Salzburg).

Concomitant interventions
Routine low-dose vitamin D supplementation (≤ 800 IU/day) is allowed during the study period and will be documented.

Adherence assessment
At visit 4 (Day 90), compliance will be assessed using the percentage of actually taken doses compared to the prescribed doses (self reported or reported by the caregivers).

Active vitamin D metabolites
Although in the future it will be interesting and necessary to assess the potential need for additional active vitamin D in critical illness, and specifically acute/chronic kidney failure, we choose not to do so in this trial because of simplicity and costs.

STEERING COMMITEE
The steering committee consists of the PI and the co-investigators.

VISIT PLAN / STUDY PROCEDURES

SCHEDULE OF EVALUATIONS

ASSESSMENT	Screening VISIT -1	Enrollment Baseline data, loading dose VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5
	Day -9 to Day 0	Day 0	Day 5	Day 28	Day 90	Month 12
Time window (days)			±2		±14	±30
25(OH)D	X					
inclusion/exclusion criteria	X	X				
signed informed consent if possible, otherwise deferred/surrogate	X	X				
randomisation		X				
demographics		X				
SAPS III		X				
TISS 28		X				
Charlson comorbidity index		X				
Stool sample (microbiome, subgroup) (optional, for Medical University Graz centers only)		X	X			
SAFETY EVALUATION						
Serum calcium	X	X	X	(X)		
Falls					X	
Fractures					X	
New episodes of nephrolithiasis					X	
Intervention						
Loading dose 540,000 IU		X				
Daily dose 4,000 IU			X	X	X	
Outcome variables						
Mortality			X	X	X	X
SOFA		X	X			
qSOFA		X				
Number of organ failures		X	X			
Infections requiring antibiotics					X	
Hospital and ICU readmission					X	

Discharge disposition					X	
Katz Activity of Daily Living		X			X	
25(OH)D (optional, for Medical University Graz centers only)		X	X			
1,25(OH)2D (optional, for Medical University Graz centers only)		X	X			

VISIT -1 (SCREENING)

25(OH)D screening is performed within clinical routine of the trial sites. Screening should be performed within the first 72 hours after ICU admission, and 25(OH)D routine testing should be available within 72 hours. After a patient has been identified to be eligible for the trial, the study medication should be given within 72 hours (max. 9 days).

If the major inclusion criterion of severe vitamin D deficiency is met, the other inclusion and exclusion criteria are evaluated by the local investigator/s at Visit 1. Informed consent will be ascertained whenever possible.

BASELINE ASSESSMENTS

- Age
- Sex
- ICU admission diagnosis
- ICU type
- Charlson comorbidity index
- SAPS III
- TISS 28
- 25(OH)D routine testing
- qSOFA
- Katz Activities of daily life

At baseline, data on demographic and clinical characteristics of the patients are obtained. At Centers of Medical University Graz it is planned to take a blood sample before the loading dose on day 0 and day 5 to ascertain biochemical response. The samples will be stored frozen at -70 C until batch analysis. In this subgroup (Medical University Graz), also stool samples will be collected for microbiome analysis on day 0 and day 5 . Stool samples are stored at -80°C

The Charlson comorbidity index, SAPS III and TISS 28 will be determined to be able to adjust for severity of illness and preexisting comorbidities.

RANDOMIZATION AND BLINDING

Patients will be randomly assigned to either placebo or vitamin D3 in a 1:1 ratio, using the web-based randomization service "Randomizer for Clinical Trials" developed at the Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz. Patients will be stratified according to trial site (ICU) and gender. An independent statistician will set up the study in the Randomizer.

The following method will be used to maintain the blind: the randomization list from the randomizer.at will be kept strictly confidential and no routine vitamin D testing is done after study inclusion. The independent statistician and unblinded pharmacist will keep treatment allocation information confidential until database lock.
In case of safety concerns (eg. severe hypercalcemia > 3.5 mmol/L), participants of the study may be unblinded by the local investigator at each participating site and/or the coordinating center. This will be done and documented with the Randomizer.

VISIT 1

Study medication
The day of the study medication loading dose is day 0. This is also the start for the calculation of the time dependent outcome data.

VISIT 2 (DAY 5 ±2)

At day 5, extensive clinical and laboratory data will be collected, including several measures of severeness of morbidity. It is likely that the majority of all patients will still be hospitalized. In the unlikely case a patient will be discharged before day 5 the data will be collected at discharge (day 3 or day 4).

VISIT 3 (DAY 28)

At visit 3, the primary endpoint (28-day mortality) will be assessed. For Austria, this information may be collected from Statistik Austria. Otherwise, this information will be obtained by the research team including nurses through the patient data management system and/or telephone.

VISIT 4 (Day 90±14d)

A follow up visit will be done by telephone 90 days after study inclusion by each study center: 1) family physician and/or 2) patient and/or 3) hospital). This visit will include important safety evaluations and secondary outcomes. This is also the end of the active intervention.

VISIT 5 (1 year ±1 month)

A final follow up visit (with results reported separately) will be done by telephone after 1 year. For Austria, Statistik Austria will be contacted first.

COMPLETION

The database lock will take place after the 90-day follow up of the last patient has been completed, all queries have been sufficiently well adressed and implemented in the database (applicable for the interim and the final analysis).

UNBLINDING FOR INTERIM AND FINAL ANALYSIS

For the formal planned interim analysis the DSMB statistician will get the unblinding list from the independent statistician responsible for the randomization procedure.
For the final analysis the unblinding list will be given to the study statisticians after database lock.

SAFETY ASSESSEMENTS

SAFETY MONITORING (SERUM CALCIUM, RENAL FUNCTION, FALLS AND FRACTURES)

Because of extremely high event rate in ICU patients and the well known risk profile of vitamin D3, safety monitoring will be restricted to known vitamin D-related adverse events.

Calcium and creatinine will be monitored regularly for routine purposes during ICU stay and recorded in the eCRF. Patients will be asked for new episodes of nephrolithiasis, falls and fractures at the 90-day-follow up telephone visit.

ADVERSE EVENT REPORTING

Only potential study drug–related adverse events (hypercalcemia, new episodes of nephrolithiasis, falls, and fractures) will be monitored and recorded up to 90 days.
Details will be specified in a separate document (SOP Reporting of SAE in intensive care – version June 2016. No separate reporting of rehospitalization or death will be performed because of the expectedly high event rates in the setting of critical illness.

STOPPING RULES FOR THE TRIAL OVERALL

A DSMB will monitor trial progress and safety. Should safety concerns evolve, the DSMB might recommend stopping the study at any time. There will be one planned interim analysis for efficacy. In case of overwhelming benefit the DSMB can recommend stopping the trial.

DATA SAFETY MONITORING BOARD (DSMB)

- ➔ Peter Suter, Prof. em., Critical Care, University Hospital Geneva
- ➔ Heike Bischoff-Ferrari, Prof., Department of Geriatrics, University of Zurich
- ➔ Martin Posch, Department of Statistics, Medical University of Vienna

STATISTICAL CONSIDERATIONS

The statistical analysis here presented will be detailed in a Statistical Analysis Plan (SAP). Should the SAP and this protocol differ, the methods in the SAP prevail. The SAP will be finalized before database lock for the planned interim analysis.

DEFINITION OF ANALYSIS SETS

Study participants who do not provide an informed consent after regaining consciousness and refuse to provide any more information are excluded from the study and will not be included in any statistical analysis.

INTENTION-TO-TREAT POPULATION

The primary analysis will be performed on the intention-to-treat population (ITT). The ITT will include all patients who receive at least the loading dose of the study medication.
All patients included here will be analysed according to the treatment assignment during randomisation.

PER PROTOCOL POPULATION

The per protocol population will include all patients who received the loading dose and have a compliance > 80%. Compliance is defined as self reported percentage of doses ingested until day 90. Other major protocol violations may also lead to exclusion of patients from the per protocol population. This will be discussed on an individual basis within the study team.

SAFETY POPULATION

The safety analyses will be based on the treated set, which is defined as all randomized patients who receive at least one dose of trial medication. All patients will be analysed according to the treatment they actually received.

DATA ANALYSIS

General aspects

All clinical and safety data collected in the study will be analysed with SAS v9.4 procedures in a Windows XP environment. Data will be presented as summary tables and, where appropriate, as plots. Continuous data will be described by means, standard deviations, medians and upper and lower quartiles unless otherwise stated. The number of observations and minimum and maximum values are also included. All descriptive summaries will be displayed to one more decimal place than actually measured. Categorical data will be summarized using frequencies and percentages.

Demographic and Baseline Characteristics

In a summary of demographic, baseline and diagnostic characteristics (age, weight, height, sex, SAPS III, Charlson comorbidity index, qSOFA - criteria, laboratory parameters,…) a comparison of the treatment groups will take place. To this end, appropriate descriptive and inferential statistics will be applied. Relevant medical history will be also displayed using summary statistics according to the two treatment groups.

Analysis of primary outcome

The primary outcome will be 28-day mortality defined as time from application of the study medication loading dose (day 0) to day 28 or death. Kaplan Meier estimates of survival curves in each treatment arm will be displayed. Group comparison will be made using a two-sided log rank test. Additionally, a hazard ratio with a 95% confidence interval will be computed from an univariate Cox-proportional hazards regression. Furthermore sensitivity analyses using multivariable Cox-proportional hazards regression will be performed adjusting for important clinical parameters. Details will be defined in the SAP.

Analysis of secondary outcomes

ICU, hospital mortality, 90-day mortality and 1-year mortality will be analysed as the primary outcome. ICU and hospital length of stay are defined as time between application of the study medication (day 0) and discharge at the primary ICU/hospital; readmissions and stays

at other hospitals will not be added, date and hour will be recorded, in case of a missing hour, the time 12am will be used.

At day 5 the SOFA Score is recorded. Organ failures (> 2 SOFA points in each of the 6 categories) will be reported in total and for each organ system separately. To compare the laboratory parameters 25(OH)D and 1,25(OH)2D levels ANCOVA will be used.

At day 90, Katz Activities of Daily Life Score, infections requiring antibiotics and hospital and ICU readmission will be assessed. The Self – reported infections requiring antibiotics and hospital and ICU readmission will be categorized as yes/no. Additionally, the number of infection episodes, number of antibiotics and -discharge disposition (home, rehabilitation, other hospital) will be presented. Comparison between groups will be performed using non-parametric tests and Chi-square tests.

Stool samples

In a subgroup, stool samples will be collected for microbiome analysis on day 0 and day 5. Stool samples are stored at -80°C. DNA extraction from stool samples will be performed by mechanical lysis with a MagnaLyser Instrument (Roche Diagnostics, Mannheim, Germany) and subsequent total bacterial genomic DNA isolation with the MagNA Pure LC DNA Isolation Kit III (bacteria, fungi) in a MagNA Pure LC 2.0 Instrument (Roche Diagnostics) according to the manufacturer's instructions. For amplification of bacterial 16S rRNA the template-specific sequence AGAGTTTGATCCTGGCTCAG and CTGCTGCCTYCCGTA, targeting the hypervariable region V1-V2 of the 16S rRNA gene, are used. PCR reactions for each sample are performed in triplicates. Subsequently the amplicons are purified according to standard procedures, quantified, pooled and sequenced with the MiSeq Reagent Kits v3 (600 cycles, Illumina, Eindhoven, Netherlands) according to manufacturer's instructions with 20% OhiX (Illumina). The generated FASTQ files are used for microbiota analysis.
Raw reads from Illumina MiSeq are pre-processed and filtered using MOTHUR v.1.31. Reads are de-noised using PyroNoise and chimera-filtered with UCHIME. Pyrosequencing errors are reduced with pre.cluster and non-bacterial sequences were also excluded. High quality reads are aligned to the SILVA database and taxonomy was assigned by MOTHUR's implementation of the ribosomal database project (RDP)-classifier followed by binning into phylotypes based on taxonomy. The shared file is then converted into a biom table and passed on to QIIME's (v.1.9.1) core_diversity.py command using non-phylogenetic parameters.

Analysis of safety outcomes

The safety outcomes, (hypercalcemia on day 5, new kidney stones, self-reported falls, and fractures until day 90) will be analysed as binary variables and compared with Chi-square tests.

Subgroup analysis

Predefined subgroup analyses will be performed for all primary and secondary outcomes based on the following group definitions as exploratory analysis:

- Kidney function (CKD 4 or lower vs. higher at inclusion)
- Sepsis (admission diagnosis) vs. non sepsis defined by the 2016 criteria (suspected infection/qSOFA on day 0 – respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less (57)

MISSING DATA HANDLING

All available data will be used in the analyses and data summaries. There will be no imputation of any missing data.

SAMPLE SIZE AND INTERIM ANALYSIS

Sample size considerations

The sample size is based on the primary endpoint 28-day mortality. In the VITdAL-ICU study (2014), 28-day mortality rates of 36% (37/102) in the placebo group and 20% (20/98) in the Vitamin D Group were observed (51). In this multicenter study we assume 28-day mortality rates of 25% in the placebo group versus 20% in the vitamin D group. We assume that baseline (placebo) mortality will be lower in this study because in contrast to the previous VITdAL-ICU study, academic and non-academic smaller sites with patients with a lower severity of illness will participate.

Using a fixed sample size design and a two-sided log-rank test for equality of survival curves with a two-sided alpha level of 5%, a sample size of n=1093 per group will be needed to achieve a power of 80% (total sample size of 2186). The **Table** below shows the sample sizes when the assumptions about 28-day mortality rates are varied between 25% - 35% for the Placebo group and between 20% - 30% for the vitamin D group as well as when the observed 28-day mortality rates from Amrein et al (2014) are assumed.

Placebo 28-day mortality	**25%**		**30%**		**Based on the VITdAL-ICU study**
Alpha level	0.05	0.05	0.05	0.05	0.05
Placebo proportion alive (π_P) at time t	**0.75**	**0.75**	0.70	0.70	**0.637**
VitD proportion alive (π_{VitD}) at time t	**0.80**	**0.81**	0.8	0.75	**0.796**
Hazard ratio $\ln(\pi_{VitD})/\ln(\pi_P)$	0.78	0.73	0.63	0.81	0.51
Power (%)	**80**	**90**	80	80	**80**
n per group	1093	1002	296	1245	129
Total number of events required	486	434	143	679	68
N Total	**2186**	**2004**	592	2490	**258**

Table: Different sample size scenarios using a two-sided log rank test

One interim analysis will be conducted when 50% of planned enrolled patients in each arm have completed their day 28 assessment (Visit 3) or prematurely discontinued the study. This interim analysis is intended to test for efficacy, i.e. the trial will be terminated after the interim analysis, if the main question can already be answered at this interim analysis.

Using a O'Brien-Fleming spending function (60) a total sample size of at least N=2194 (494 events) is required to achieve 80% power. With this sample size a hazard ratio of 0.78 (for survival rates of 0.8 in the Vitamin D group and 0.75 in the Placebo group corresponding to a 5% absolute 28-day mortality difference) can be detected, using a 2-sided log rank test with 0.05 alpha level assuming that the hazards are proportional. Accounting for a drop-out rate of approximately 10% yields a total sample size of N=2400 patients. A **power of 90%** would be achieved with a similar sample size if the treatment effect is larger (Hazard Ratio 0,73, or a 6% absolute 28-day mortality difference).

Planned interim analysis

One formal interim analysis by the Data Safety Monitoring Board (DSMB) will be performed at inclusion of 50% (N=1200) of patients having their day 28 assessment completed or discontinued the study. The interim analysis will take place approximately 12-18 months after start of the study. If the interim analysis shows a benefit for the vitamin D group, the DSMB may recommend early study termination.

The interim analysis will be performed only for the primary outcome 28 day mortality. The O' Brien-Fleming rule will be used to stop the trial early for efficacy. In detail, if the p-value of the log rank test is smaller than 0.003, then the trial can be stopped early by the DSMB.

Only the primary endpoint will be assessed for the interim analysis with an alpha level of 0.003. It is planned to test the secondary efficacy variables and the safety outcomes at an alpha level of 0.05. We will specify this and any deviations from the study protocol in the SAP.

DATA COLLECTION AND QUALITY ASSURANCE/DATA MANAGEMENT

DATA HANDLING

Every center will use a specific abbreviation (eg. Salzburg – SBG). Individual patients will be identified using a number (eg. First patient in Salzburg: SBG – 001).

DATA COLLECTION FORMS - ELECTRONIC CRF

Every center will have access to the electronic centralized case report form *Clincase* (Quadratek Data Solutions Ltd., Berlin, Germany). *Clincase* is a validated EDC (electronic data capture) and CDM (clinical data management) system for all types and phases of clinical trials and registries. It complies fully with FDA 21 CFR part 11 and EU GMP Annex 11 regulations. A backup paper version will be available to all centers.

QUALITY ASSURANCE AND MONITORING

Monitoring and audits shall be performed during the clinical trial for the purpose of quality assurance. Details will be specified in the Monitoring Manual.

ETHICAL CONSIDERATIONS AND COUNTRY-SPECIFIC INFORMED CONSENT PROCEDURE

The study will be performed according to national laws, the Declaration of Helsinki and current ICH-GCP guidelines and will be submitted to the Ethics Committees of each participating country.

INFORMED CONSENT PROCEDURE

One important aspect in critically ill patients is informed consent. The majority of patients will not be able to give informed consent at the time of study inclusion due to altered state of consciousness. Only a minority of patients will be able to give full informed consent during the acute setting.

Country specific regulations when immediate informed consent is not possible

Whenever possible, written informed consent will be obtained directly from the patient or from a legal surrogate. The majority of patients, however, will not be able to give informed consent due to acute illness (e.g. sepsis), intubation, mechanical ventilation and sedation. Only a minority of patients will be able to give full informed consent in the acute setting. Based on the VITdAL trial, we assume that >80% of patients will not be able to give informed consent at the time of randomization.
The following procedures will be applied:

- Patient with full consent or available legal surrogate → Immediate informed consent
- Patient not able to consent → country specific regulations as detailed below
- Patient recovers to full consent → retrospective informed consent

Patient information and informed consent will be handled according to the rules of "Good Clinical Practice" and the "Declaration of Helsinki". All eligible patients will undergo the consent process prior to randomization as described above using different forms.

Country specific regulations apply in case patient is not able to consent:

Austria:
Deferred/surrogate informed consent (IC)
The institutional ethical committee, similar to other states of the European Union, approves the use of "surrogate consent." Informed consent will be obtained at a later time point if the patient survives and regains mental capacity.

Germany:
"Konsiliararztverfahren": 1-2 independent physician/s assess/es supposed patient's will (if possible by contact of relatives).
Alternatively, a legal representative, e.g. relative or person in charge by the guardianship court, needs to be contacted after inclusion to provide informed consent.

Switzerland:
"Konsiliararztverfahren": one independent physician assesses inclusion/exclusion criteria. Additionally, a relative or person in charge by the guardianship court needs to be contacted to provide informed consent.

Other countries that may participate in this study are the Belgium, the Czech Republic, Denmark the United Kingdom and Canada. Country-specific regulations exist but are not discussed here.

REFERENCES

1. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab. 2009;94(1):26-34.
2. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29(6):726-76.
3. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.
4. Heaney RP. Assessing vitamin D status. Curr Opin Clin Nutr Metab Care. 2011;14(5):440-4.
5. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369(21):1991-2000.
6. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2011(7):CD007470.
7. Amrein K, Venkatesh B. Vitamin D and the critically ill patient. Curr Opin Clin Nutr Metab Care. 2012;15(2):188-93.
8. McNally JD, Menon K. Vitamin D deficiency in surgical congenital heart disease: prevalence and relevance. Translational Pediatrics. 2013;2(3):99-111.
9. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. BMJ. 2014;349:g6330.
10. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2014;1:CD007470.
11. Rejnmark L, Avenell A, Masud T, Anderson F, Meyer HE, Sanders KM, et al. Vitamin d with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin d trials. J Clin Endocrinol Metab. 2012;97(8):2670-81.
12. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2(1):76-89.
13. Bolland MJ, Grey A, Gamble GD, Reid IR. The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. Lancet Diabetes Endocrinol. 2014;2(4):307-20.
14. 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(2):R47.
15. Zhang YP, Wan YD, Sun TW, Kan QC, Wang LX. Association between vitamin D deficiency and mortality in critically ill adult patients: a meta-analysis of cohort studies. Crit Care. 2014;18(6):684.
16. Upala S, Sanguankeo A, Permpalung N. Significant association between vitamin D deficiency and sepsis: a systematic review and meta-analysis. BMC Anesthesiol. 2015;15:84.
17. de Haan K, Groeneveld AB, de Geus HR, Egal M, Struijs A. 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(6):660.
18. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41(2):580-637.
19. Danai PA, Sinha S, Moss M, Haber MJ, Martin GS. Seasonal variation in the epidemiology of sepsis. Crit Care Med. 2007;35(2):410-5.
20. Remmelts HH, van de Garde EM, Meijvis SC, Peelen EL, Damoiseaux JG, Grutters JC, et al. Addition of vitamin D status to prognostic scores improves the prediction of outcome in community-acquired pneumonia. Clin Infect Dis. 2012;55(11):1488-94.
21. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013;5(7):2502-21.
22. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Rheum Dis Clin North Am. 2012;38(1):125-39.
23. De Pascale G, Vallecoccia MS, Schiattarella A, Di Gravio V, Cutuli SL, Bello G, et al. Clinical and microbiological outcome in septic patients with extremely low 25-hydroxyvitamin D levels at initiation of critical care. Clin Microbiol Infect. 2015.
24. Rajakumar K, Greenspan SL, Thomas SB, Holick MF. SOLAR ultraviolet radiation and vitamin D: a historical perspective. Am J Public Health. 2007;97(10):1746-54.
25. Levine JA. Sick of sitting. Diabetologia. 2015;58(8):1751-8.
26. Perron RM, Lee P. Efficacy of high-dose vitamin D supplementation in the critically ill patients. Inflamm Allergy Drug Targets. 2013;12(4):273-81.
27. Zajic P, Amrein K. Vitamin D deficiency in the ICU - a systematic review. Minerva Endocrinol. 2014.

28. Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E, et al. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med. 2011;39(4):671-7.
29. Padhi R, Panda B, Jagati S, Patra SC. Vitamin D status in adult critically ill patients in Eastern India: An observational retrospective study. Lung India. 2014;31(3):212-6.
30. Moraes RB, Friedman G, Wawrzeniak IC, Marques LS, Nagel FM, Lisboa TC, et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics (Sao Paulo). 2015;70(5):326-32.
31. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, et al. Impact of anesthesia and surgery for congenital heart disease on the vitamin d status of infants and children: a prospective longitudinal study. Anesthesiology. 2013;119(1):71-80.
32. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360(18):1912-4.
33. Amrein K, Christopher KB, McNally JD. Understanding vitamin D deficiency in intensive care patients. Intensive Care Med. 2015;41(11):1961-4.
34. Lee P. Vitamin D metabolism and deficiency in critical illness. Best Pract Res Clin Endocrinol Metab. 2011;25(5):769-81.
35. 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(12):3170-9.
36. Thickett DR, Moromizato T, Litonjua AA, Amrein K, Quraishi SA, Lee-Sarwar KA, et al. Association between prehospital vitamin D status and incident acute respiratory failure in critically ill patients: a retrospective cohort study. BMJ Open Respir Res. 2015;2(1):e000074.
37. Dancer RC, Parekh D, Lax S, D'Souza V, Zheng S, Bassford CR, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax. 2015;70(7):617-24.
38. Quraishi SA, Litonjua AA, Moromizato T, Gibbons FK, Camargo CA, Jr., Giovannucci E, et al. Association between prehospital vitamin D status and hospital-acquired bloodstream infections. Am J Clin Nutr. 2013;98(4):952-9.
39. Quraishi SA, Litonjua AA, Moromizato T, Gibbons FK, Camargo CA, Jr., Giovannucci E, et al. Association between prehospital vitamin D status and hospital-acquired Clostridium difficile infections. JPEN J Parenter Enteral Nutr. 2015;39(1):47-55.
40. 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(1):97-107.
41. 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(1):63-72.
42. Madden K, Feldman HA, Smith EM, Gordon CM, Keisling SM, Sullivan RM, et al. Vitamin D Deficiency in Critically Ill Children. Pediatrics. 2012.
43. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, et al. The Association of Vitamin D Status With Pediatric Critical Illness. Pediatrics. 2012.
44. Amrein K, Quraishi SA, Litonjua AA, Gibbons FK, Pieber TR, Camargo CA, Jr., et al. Evidence for a U-shaped relationship between prehospital vitamin D status and mortality: a cohort study. J Clin Endocrinol Metab. 2014;99(4):1461-9.
45. Lee P, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? Intensive Care Med. 2009.
46. Vanek VW, Borum P, Buchman A, Fessler TA, Howard L, Jeejeebhoy K, et al. A.S.P.E.N. position paper: recommendations for changes in commercially available parenteral multivitamin and multi-trace element products. Nutr Clin Pract. 2012;27(4):440-91.
47. Krishnan A, 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(4):230-8.
48. 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(1):53-8.
49. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Dietary Reference Intakes for Calcium and Vitamin D. Institute of Medicine, 2011.
50. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.

51. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, 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(15):1520-30.
52. Higgins DM, Wischmeyer PE, Queensland KM, Sillau SH, Sufit AJ, Heyland DK. Relationship of vitamin D deficiency to clinical outcomes in critically ill patients. JPEN J Parenter Enteral Nutr. 2012;36(6):713-20.
53. von Restorff C, Bischoff-Ferrari HA, Theiler R. High-dose oral vitamin D3 supplementation in rheumatology patients with severe vitamin D3 deficiency. Bone. 2009;45(4):747-9.
54. Vieth R. Critique of the considerations for establishing the tolerable upper intake level for vitamin D: critical need for revision upwards. J Nutr. 2006;136(4):1117-22.
55. Landoni G, Comis M, Conte M, Finco G, Mucchetti M, Paternoster G, et al. Mortality in Multicenter Critical Care Trials: An Analysis of Interventions With a Significant Effect. Crit Care Med. 2015;43(8):1559-68.
56. Heyland DK, Stapleton RD, Mourtzakis M, Hough CL, Morris P, Deutz NE, et al. Combining nutrition and exercise to optimize survival and recovery from critical illness: Conceptual and methodological issues. Clin Nutr. 2015.
57. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-10.
58. 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(11):2313-20.
59. Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. Nutr Rev. 2014;72(1):48-54.
60. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979;35(3):549-56.

List of Abbreviations

25(OH)D	25-hydroxyvitamin D, native vitamin D
1,25(OH)2D	1,25-dihydroxyvitamin D, actie vitamin D
AE	Adverse Event
CDM	clinical data management
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EDC	Electronic data capture
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IOM	Institute of Medicine
JAMA	Journal of the American Medical Association
LKH	Landeskrankenhaus
MCT	Medium chain triglycerides
N	Number (typically refers to subjects)
NEJM	New England Journal of Medicine
PI	Principal Investigator
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
WHO	World Health Organization

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Effect of high-dose vitamin D3 on 28-day mortality in adult critically ill patients with severe vitamin D deficiency: a multicentre, placebo-controlled double-blind phase III RCT (The VITDALIZE Study) Page 1
Trial registration	2a	ClinicalTrials.gov (identifier: NCT03188796) EudraCT-No.: 2016-002460-13 Page 2
	2b	All items from the World Health Organization Trial Registration Data Set No changes and amendments.
Protocol version	3	24th of January 2018, Version 1.3
Funding	4	BMBF fund (01KG1815), ESICM, NHS fund (17/147/33), Fresenius Kabi

Roles and responsibilities	5a	Principle Investigator Austria Prof. Dr. med Karin Amrein Division for Endocrinology and Diabetology Medical University of Graz Auenbruggerplatz 15 8036 Graz, Austria Coordinating Investigator Germany Prof. Dr. med Patrick Meybohm Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy University Hospital Frankfurt Theodor-Stern-Kai 7 60590 Frankfurt am Main, Germany Coordinating Investigator Belgium Dr. med. Dr. rer. nat. Jean-Charles Preiser Department of Intensive Care Erasme University Hospital Route de Lennik 808 1070 Brussels, Belgium Coordinating Investigator UK Dr. rer. nat. Dhruv Parekh Institute of Inflammation and Ageing University of Birmingham Mindelsohn Way Birmingham, B15 2WB, UK Coordinating Investigator Switzerland Prof. Dr. med. Marco Maggiorini Institute for Intensive Care University Hospital Zurich Rämistrasse 100 8097 Zurich, Switzerland

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5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
The sponsors and funders had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Coordinating center
Design of VITDALIZE
Preparation of protocol, IB and CRFs and revisions
Publication of study reports
Steering Committee
Recruitment of patients
Reviewing progress and if necessary agreeing changes to the protocol
Trial management committee
SUSAR reporting
Responsible for trial master file
Budget administration
Data verification
Randomisation
Data management
Maintenance of trial IT system and data entry
Data verification

Data Monitoring Safety Board (DMSB)

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Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention ➔ Page 4
	6b	Explanation for choice of comparators ➔ Page 4
Objectives	7	Specific objectives or hypotheses ➔ Page 4+5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) ➔ page 2+5

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained ➔ Page 2 ➔ Page 16-21 Acknowledgement
Eligibility criteria	10	Inclusion and exclusion criteria for participants. ➔ Page 8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered ➔ Page 7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) ➔ Study protocol (Supplementary)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) ➔ Study protocol (Supplementary)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial In case of hypercalcemia immediate stopping of vitamin D administration ➔ Study protocol (Supplementary)

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended ➔ Page 8-9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Figure 1, Table 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations ➔ Page 10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size ➔ Page 6-7

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions ➔ Page 7
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned ➔ Page 8-9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions ➔ Page 7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how ➔ Page 7

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial ➔ Study protocol (Supplementary)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol ➔ Study protocol (Supplementary)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols ➔ Page 8-9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol ➔ Study protocol (Supplementary)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol ➔ Page 10-12 ➔ Study protocol (Supplementary)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) ➔ Page 11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) ➔ Page 11-12
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed ➔ Page 21-22 (Acknowledgement)

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial ➔ Page 12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct ➔ Page 9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor ➔ Not applicable

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Study protocol V1.3 from 24th January 2018 (EudraCT 2016-002460-13), patient information, and informed consent were or will approved of all participating centres.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Ethics Committees and group messages to all participating centers
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Study nurse or physicians will explain the trial to the patient and give explanation sheet to participant. Questions will be answered and discuss. Study nurse or physician will obtain written consent from patients agree to participate in the trial.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial ➔ Study protocol (Supplementary)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site YES

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators The Data Management Centre will monitor the dataset. Each Principal investigator will be given full access to the cleaned data set.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Not applicable
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions ➔ Page 2, 16
	31b	Authorship eligibility guidelines and any intended use of professional writers YES, page 2
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code yes
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates no
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.