VITdAL@ICU

Correction of vitamin D deficiency in critically ill patients

Study Protocol

Version 1.0, 19.02.2010
Original Version

Version 2.0, 10.11.2010
Addition of personal variant for 6–month follow-up (including assessment of falls and fractures) and data safety review

Addition of 24-month follow-up
**SYNOPSIS**

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| Title | Correction of vitamin D deficiency in critically ill patients: a randomized, double-blind, placebo-controlled trial (“VITdAL@ICU”) |

| Background | Low vitamin D status is associated with increased mortality, cardiovascular events, diabetes, hypertension and impaired function of the immune and musculoskeletal system in cross-sectional and prospective cohort studies. Given that most critically ill patients are vitamin D deficient, treatment with sufficiently high doses of vitamin D may represent a promising and inexpensive intervention option. To date, no clinical trial has prospectively evaluated clinical outcomes in patients treated with vitamin D in an intensive care setting. |

| Objectives/Outcome measures | Primary endpoint  
| - hospital stay  
| Secondary endpoints  
| - ICU stay  
| - mortality in ICU, in hospital, at day 28 and at 6 months  
| - percentage of patients with 25(OH)D ≥ 30 ng/ml at day 7  
| - laboratory parameters including serum calcium, 25(OH)D at days 0,3,7 and 28 (if the patient is still hospitalised)  
| - duration of mechanical ventilation  
| - cardiac function (NT-proBNP levels, days on catecholamine support)  
| - TISS 28 scores during ICU stay |

| Study design | Randomized, placebo-controlled, double-blind, single centre study |

| Project schedule | Study related  
| Planned start of recruitment: April 2010  
| Active enrolment period: 24 months  
| Subject related  
| Total study duration for each patient will be 28 days with a follow up |
### Study population
Adult patients admitted to medical, surgical and neurological intensive care units of the university hospital of Graz, Austria

### Trial site
Medical University of Graz, Austria

### Sample size
480 patients (240 in each group)

### Inclusion criteria
- Age ≥18 years
- expected ICU stay ≥48 hours
- vitamin D deficiency: 25(OH)D ≤ 20 ng/ml

### Exclusion criteria
- hypercalcaemia (total calcium >2.65 OR ion. calcium >1.35 mmol/l)
- severely impaired gastrointestinal motility (ileus, residual gastric volume > 400 ml)
- known history of recent kidney stones (≤ 1 year)
- known granulomatous diseases (tuberculosis, sarcoidosis)
- pregnancy/lactation
- not suitable for other reasons (living abroad etc.)

### Study procedures
Screening for vitamin D deficiency immediately after admission to ICU, randomisation, application of first study drug as soon as inclusion and exclusion criteria are available (day 0)

### Study related investigations
Day 0, 3, 7 and 28 if feasible:
Most parameters (i.e. standard blood chemistry including serum creatinine and albumin levels, serum calcium, phosphate, magnesium, CRP, PCT, NT-pro BNP levels and urinary calcium, 25(OH)D, 1,25(OH)2D, PTH, bone turnover markers) will be measured immediately. Other parameters such as IL-6 and cathelicidin levels may be measured at the end of the study from frozen blood specimens.
Information on SAPS II and TISS 28 scores of participants as well as vital status and morbidity will be derived from source data within the medical documentation system at the hospital (MEDOCS). In case of patient discharge from the hospital, vital status will be assessed by a telephone visit at days 28 and month 6.

### Investigational product
**Intervention:** cholecalciferol (trade name: Oleovit® D3)
**Manufacturer:** Fresenius Kabi Austria GmbH

### Study treatment
**Investigational product:** cholecalciferol
**Comparator drug:** Placebo identical in colour, smell and texture
**Dose:** one single dose of 540,000 IU vitamin D dissolved in a volume of
| Herbal oil of 45 ml OR same volume of oil (placebo). This loading dose regimen will be followed by monthly doses of 90.000 IU D (corresponding to 3000 IU/day) or respective placebo up to month 6. |
| Route of administration: enteral via nasogastric tube (ICU setting) or oral (if possible) |
| Duration of treatment: 5 months |
Introduction

Objective

The primary objective of this study is to investigate whether oral high-dose vitamin D supplementation is beneficial for vitamin D deficient, critically ill patients and whether the length of hospital stay is affected.

Background and current status of research

Cutaneous synthesis is the main source of vitamin D as opposed to only small quantities coming from oral intake. Thus, homebound, hospitalised and elderly individuals are prone to develop vitamin D insufficiency but in many instances a low vitamin D status is found in healthy individuals as well. According to our own and international observations, prevalence of vitamin D deficiency and insufficiency is especially high in patients at intensive care units [1, 2]. Insufficient vitamin D levels could affect critically ill patients in various ways [3].

Hundreds of genes with vitamin D receptor response elements directly or indirectly influence cell cycling, cell proliferation, differentiation and apoptosis suggesting a much more widespread function than previously thought [4]. The vitamin D receptor (VDR) is almost ubiquitously expressed, and the vast majority of cells respond to 1,25(OH)2D2 exposure. VDR-deficient mice develop high renin hypertension, cardiac hypertrophy, and show increased thrombogenicity among other phenotypes. Vitamin D deficiency in humans is associated with an increased prevalence of a multitude of diseases [5].

Vitamin D and glucose metabolism

Glucose metabolism is impaired in many critically ill patients and is often aggravated by parenteral feeding, infections and/or pre-existent diabetes. Contributing mechanisms are elevated hepatic glucose production and dysregulated peripheral glucose uptake accompanied by insulin resistance [6]. This well-appreciated phenomenon was named “diabetes of injury” or “stress diabetes” [7, 8].

Vitamin D seems to act favourably on glycemic control [9] and a recent trial has shown that baseline 25(OH)D was inversely correlated to the 10-year risk of incident hyperglycemia [10]. These findings were confirmed by a recent Finnish study showing an inverse association between baseline 25(OH)D and 17-year risk of type 2 diabetes [11]. Moreover, glycemic control in diabetic subjects varies throughout the year, with lower HbA1c concentrations occurring in summer [12]. Whether vitamin D influences insulin resistance, secretion or both still has to be elucidated by performing glucose clamp studies.

Vitamin D and cardiovascular aspects

A growing body of data suggests that low vitamin D levels may adversely affect cardiovascular health. For many cardiovascular events, seasonal variability with peak incidence in the winter months is proven. This may be attributable at least in part to declining body stores of vitamin D beginning with September [13]. Recently, there have been several case reports about severe cardiomyopathy caused by vitamin D deficiency, especially in dark-skinned children who had low vitamin D levels [14].
The heart is an important target organ for vitamin D, both on a genomic and nongenomic level. Myocytes express the vitamin D receptor and several models of hypertension in animal studies have shown that vitamin D treatment is able to prevent cardiac hypertrophy [15, 16]. Vitamin D seems to inhibit activation of the cardiac renin-angiotensin system as well as the expression of genes involved in the development of myocardial hypertrophy. There is accumulating evidence that vitamin D deficiency may be an important factor in the development of congestive heart failure and sudden cardiac death [17].

In chronic hemodialysis patients, vitamin D supplementation has been associated with reduction of cardiac hypertrophy and a reduction of QT dispersion [18], the latter being considered a major risk factor for sudden cardiac death.

A recent study from our group has reported a negative correlation of 25(OH)D levels with NT-pro-BNP levels, New York Heart Association functional classes and impaired left ventricular function [17]. Furthermore, hazard ratios for death attributable to heart failure and sudden cardiac death were 2.84 and 5.05, respectively, when patients with 25(OH)D <25ng/ml were compared with those having serum levels of 25(OH)D >75 ng/ml [17]. The anti-inflammatory properties of vitamin D also appear to play a role in congestive heart failure, as studied in a recent interventional trial [19]. In animal models, vitamin D deficiency was proven to be associated with developing myocardial hypertrophy and fibrosis with aberrant cardiac contractility and relaxation [15, 16].

Moreover, vitamin D deficiency can raise parathyroid hormone secretion, which in turn may increase insulin resistance and be associated with the development of diabetes, hypertension and inflammation.

In summary, vitamin D seems to exert a multitude of different effects all working in concert to protect the vascular and cardiac system by influencing various hierarchical levels of biologic response.

**Vitamin D and mortality**

Several studies from our group have recently reported associations between low baseline 25(OH)D levels and adverse cardiovascular outcomes, stroke, increased cancer and all-cause mortality in a large prospective cohort of patients at increased cardiovascular risk [20-25]. In these studies, all-cause mortality was independently related to low 25(OH)D as well as to low 1,25(OH)2D levels regardless of average physical activity levels and degree of present comorbidities.

In a recent study that analyzed data of more than 16,000 patients on hemodialysis, oral supplementation with vitamin D was found to improve overall survival. In this study, type 2 diabetes was the cause for end-stage renal disease in almost a third of the patients [26]. Interestingly, a recent meta-analysis including trials mainly designed to investigate the effects of vitamin D on bone mass development or fracture occurrence demonstrated a significant reduction in all-cause mortality with doses of vitamin D that today are thought to be inadequately small [27].

**Vitamin D and infection**

Most cells of the immune system express the vitamin D receptor [5]. Vitamin D acts on immune function, both on adaptive and innate immunity. It enables macrophages
to respond to and kill bacterial and viral organisms and exerts an influence on epidermal keratinocytes to respond to disruption of barrier function [28]. Antimicrobial peptides such as cathelicidins contribute to initial defense of the airway system against inhaled pathogens. Recent studies have shown that the hormonally active form of vitamin D3, 1,25(OH)2D upregulates antimicrobial gene expression in several established cell lines. Interestingly, treatment of normal human bronchial epithelial cells with 1,25(OH)2D resulted in a 10-fold up-regulation of cathelicidin mRNA levels after 12 hours [29]. In a recent study, the association between 25(OH)D levels and occurrence of upper respiratory tract infection was examined in a large cross-sectional sample that represented the entire US population [30]. Individuals had multivariate adjusted OR of between 1.24 (95%CI 1.07-1.43) to 1.36 (1.01-1.84) depending on whether they had serum 25(OH)D levels between 10 to 30 ng/ml or <10 ng/ml respectively.

Vitamin D has also been implicated to induce endogenous antimicrobial peptides in the epithelium of the gastro-intestinal tract through induction of LL-37, an important cathelicidin of the large intestine. These enterocyte-derived antimicrobial peptides are secreted into a thin film overlying the luminal surface of the intestine, forming an antimicrobial barrier that discourages the successful attachment and growth of microbes within the lumen [31].

It is well known that maintenance of an intact epidermal and epithelial barrier function is of special clinical concern in critically ill patients because these patients are at high risk of developing nosocomial infections of the respiratory and gastrointestinal tract that often lead to septicemia.

In summary, based on the presented data we hypothesize that increasing 25(OH)D levels will help critically ill patients to better fight nosocomial infections or even allow to prevent an outbreak of infection.

**Vitamin D and clinical aspects of patients at the ICU**

Vitamin D deficiency is a highly prevalent condition affecting approximately 30% to 50% of the general population and up to 90% of critically ill patients [2, 32]. Similar percentages of patients with low vitamin D status could be substantiated at our general internal wards (median 25(OH)D level of 16 ng/ml) as well as at intensive care units (median 25(OH)D level of 13.2 ng/ml) (unpublished data).

The daily amount of vitamin D recommended for parenteral nutrition is approximately 200 IU. However, even a dose of 600 IU can be considered too low for patients with established vitamin D deficiency in order to establish a normal 25(OH)D status. Such patients first require a loading dose to saturate adipose tissue stores throughout the body if one intends to restore serum 25(OH)D quickly. At a second step higher than currently used daily doses of vitamin D3 should be given to patients to guarantee adequately high serum concentration and thus substrate concentration for the many cell types metabolizing 25(OH)D.

Of utmost interest to the proposal of this study is a previous report where hypocalcemia and elevated PTH levels were found to be important determinants of elevated morbidity and mortality in patients at ICU [33]. In addition, vitamin D deficiency can contribute to the development of hypophosphatemia which is also a highly frequent condition encountered in the critically ill, affecting 30-45% of all patients [34, 35]. Hypophosphatemia also has been associated with adverse outcomes in several studies [34].

We propose that a potential least common denominator of all the described findings (hyperparathyroidism, hypocalcemia, hypophosphatemia) is a low vitamin D status that could be causally linked to the observed increase in mortality of ICU patients.
Muscle wasting is another clinical consequence of vitamin D deficiency, which increases the risk for prolonged mechanical ventilation and causes difficulties during the weaning process. Severe vitamin D deficiency is associated with myopathy, decreased muscle strength and an increased risk for falls [5]. In randomized controlled trials, low-dose vitamin D supplementation was shown to affect muscle strength in elderly patients with vitamin D deficiency [36] and decreased the frequency of falls [36-38]. Vitamin D treatment possibly via genomic and non-genomic effects leads to better muscle contraction and performance which could be of potential interest for the patient in the intensive care setting.

**Justification of high-dose vitamin D3 intervention**

The longer half-life of 25(OH)D following oral cholecalciferol supplementation allows for larger doses to be administered because they are a practical alternative to frequent daily dosing. Large loading doses of vitamin D3 have been shown to rapidly and safely normalize 25(OH)D levels in elderly patients with vitamin D deficiency (defined as below 20ng/ml) [39]. Smaller doses of vitamin D3 are similarly effective but require a much longer period until a plateau of 25(OH)D levels can be reached.

A loading dose of 500,000 IU of vitamin D raised 25(OH)D levels from 14 ng/ml to 40 ng/ml four weeks after administration [40]. Thereafter, levels declined to mean levels below 30ng/ml over the next 8 months. The best treatment regimen in that study consisted of a loading dose of 500,000 IU at baseline which was followed by the administration of 90,000 IU per month (corresponding to 3000 IU/day) for 8 more months. Even when giving such a loading dose to patients with 25(OH)D levels >20ng/ml, maximum mean 25(OH)D levels did not rise above 56 ng/ml. There was no safety issue in the reported study and serum calcium values were all within the reference range.

Several other studies have also assessed the effects of large intermittent doses between 300,000 and 600,000 IU of vitamin D and found them to be safe [41-43]. Vieth has recently reviewed the issue of “vitamin D toxicity” and concluded 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 [44].

**Outcome Variables**

Because of restricted financial and personal resources, we chose to rather determine surrogate parameters that can easily be collected and that reflect overall health status, cardiac performance, or episodes with infections rather than more specific outcome related variables which may be more examiner-dependent and/or costly to obtain (i.e. cardiac ultrasound).

**Primary endpoint**
- hospital length of stay

**Secondary endpoints**
- ICU stay
- overall mortality in ICU, in hospital, at day 28 and at 6 months
- percentage of patients with 25(OH)D ≥ 30 ng/ml at day 7
- calcium; phosphorus; 25(OH)D; 1,25(OH)D; PTH; urinary calcium at days 0,3,7,28 (if feasible)
VITdAL@ICU - Correction of vitamin D deficiency in critically ill patients

- CRP- and PCT-levels TISS 28 scores during ICU stay
- duration of mechanical ventilation
- cardiac function (NT-proBNP levels, days on catecholamine support)

Methods of procedure

Serum 25(OH)D will be measured in patients admitted to the intensive care unit (day 1) and fulfilling the inclusion criteria. Patients in the cardiothoracic surgery unit may be asked for informed consent preoperatively if ICU stay is anticipated to last for 48 hours as for example after double valve replacement surgery. After the patient has been informed about the trial and written informed consent obtained, a screening number will be assigned to the patient in ascending order.

For patients not currently able to give informed consent, the enrolment procedure will be discussed with the Ethical Committee of our institution before commencement of the trial. In view of the safety of vitamin D loading dose regimens, we anticipate that inclusion of patients unable to sign informed consent will be granted until the patient is able to provide informed consent. At this stage, the patient can decline further study participation and will not be considered in the analysis.

Following inclusion, each patient will receive either an oral cholecalciferol loading dose of 540,000 IU or placebo in matching volumes.

Individuals will be randomised to vitamin D or placebo using a web-based randomisation tool (http://www.randomizer.at).

Routine care will be provided for each patient. Trial-related activities will not interfere with regular patient care.

Statistical aspects/Power analysis/Sample size calculation

We based our sample size calculation on data for hospital stay (starting at ICU admittance) in 2008 for critically ill patients who had stayed ≥ 48h on the concerned intensive care units (medical, cardiothoracic, neurologic intensive care unit, coronary care unit).

Using the Mann-Whitney U-Test and a logarithmic model for sample size calculation, a group size of 234 is needed to show a reduction of 2 days (equivalent to 14%) of the primary study outcome (based on hospital stay of 14 days, a standard deviation of 7 days, alpha of 0.05 and statistical power of 0.80).

Only few patients are expected to withdraw consent and thus drop-out of the study, so approximately 240 patients will need to be enrolled in each group.

Study design

- Prospective, double-blind, randomized, placebo-controlled trial
- Trial site: several ICUs Medical University Graz
- Study population: patients admitted to the participating ICUs
- Intervention: high-dose vitamin D3 (cholecalciferol) or placebo

Study duration

The active treatment period for each patient will be 7 days with an optional follow-up at day 28 and with a follow up telephone visit at 6 months. Total recruitment period will last for approximately 24 months. Thus total study duration will be 30 months.
Inclusion criteria
- age ≥18 years
- expected ICU stay ≥ 48 hours
- vitamin D deficiency: 25(OH)D ≤ 20 ng/ml

Exclusion criteria
- hypercalcemia (total calcium >2.65 mmol/l OR ionized calcium >1.35 mmol/l)
- severely impaired gastrointestinal function (ileus, residual gastric volume > 400 ml)
- known granulomatous diseases (tuberculosis, sarcoidosis)
- known history of recent kidney stones (≤1 year)
- pregnancy/lactation
- patients not deemed suitable for study participation (i.e. psychiatric disease, prisoner status, living abroad/remote from the clinic, moribund condition at screening)

Women of childbearing age – pregnancy tests
Women of childbearing age are only allowed to participate in this trial when pregnancy has been excluded by testing.

Ethical aspects
Vitamin D treatment is simple, safe and inexpensive. The importance of treatment with sufficiently high doses of cholecalciferol has been undervalued for the longest time. Based on what is known already, vitamin D affects the cardiovascular system, glucose metabolism, immunocompetent cell systems, the musculoskeletal system and regulates cell cycle.

So far, no randomized controlled trial has explored the potential of vitamin D treatment for decreasing adverse outcomes related to cardiovascular disease and overall health in ICU patients. Despite the high prevalence of vitamin D deficiency in critically ill patients, no interventional trial has so far been performed in this setting.

Data safety review
We aim to have a data safety review meeting after 1 year or 100 patients.
References

Version 2.0, 10.11.2010
Addition of personal variant for 6 – month – follow-up (including assessment of falls and fractures) and data safety review

6 – Month – Follow-Up

- Variant A (ca. 80%): follow-up by telephone, CRF Month 6
- Variant B (ca. 20%): personal follow-up
  - Blood sample as on day 0,3,7,28
  - Timed Up & Go - Test
  - DXA with bone mineral density and body composition, radiation dose: approx. 10 μSievert
  - CRF Month 6

Data safety review
A first data safety review will be performed after 100 patients, a second after 250 patients.
Addition of 24-month follow-up

24 – Month – Follow-Up

- Variant A (ca. 80%): follow-up by telephone, CRF Month 24
- Variant B (ca. 20%): personal follow-up
  - Blood sample as on day 0,3,7,28
  - Timed Up & Go - Test
  - DXA with bone mineral density and body composition, radiation dose: approx. 10 μSievert
  - CRF Month 24
Statistical Analysis Plan

Study Number: ClinicalTrials: NCT01130181

Study Title: Correction of vitamin D deficiency in critically ill patients: a randomized, double-blind, placebo-controlled trial ("VITDAL@ICU")

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Statistical Analysis Plan

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1. Introduction

Low vitamin D status is associated with increased mortality, cardiovascular events, diabetes, hypertension and impaired function of the immune and musculoskeletal system in cross-sectional and prospective cohort studies. Given that most critically ill patients are vitamin D deficient, treatment with sufficiently high doses of vitamin D may represent a promising and inexpensive intervention option. To date, no clinical trial has prospectively evaluated clinical outcomes in patients treated with vitamin D in an intensive care setting.

The hypothesis of this first large RCT is that supplementation with a sufficiently large dose of vitamin D leads to a fast correction of low vitamin D status and possibly to clinical benefits, particularly relating to immune, cardiac and muscle function. Because these potential benefits likely occur at various functional hierarchies, we chose "length of hospital stay" as a primary surrogate marker for a change in morbidity in this patient group.

This document describes the analytical and statistical methods that will be employed.

2. Study Objectives

The primary objective of this investigator-initiated, randomized, double-blind, placebo-controlled trial is to assess the effect of oral high-dose vitamin D supplementation in comparison to placebo treatment on length of hospital stay in vitamin D deficient, critically ill patients.

3. Study Design

The study is performed as an investigator-initiated, randomized, double-blind, placebo-controlled single-centre, two-arm parallel group study comparing oral high-dose vitamin D supplementation to placebo-treatment in vitamin D deficient, critically ill adult patients. Following an initial loading dose of 540,000 IU of vitamin D3, patients receive 90,000 IU of vitamin D3 on a monthly basis for 5 months or corresponding placebo resulting in a total treatment/observation period of 6 months. A total of 480 critically ill patients with low 25(OH)D levels (≤ 20ng/ml) admitted to 5 intensive care units of the University Hospital of Graz, Austria will be enrolled. It recruits patients at the following ICUs: medical (15 beds), neurological (8 beds), cardiothoracic surgery (13 beds) and two mixed surgery units (12 and 10 beds).

Upon admission to one of the five participating ICUs, all adult patients undergo screening for study participation. Patients ≥18 years who are expected to stay in ICU ≥48 hours and who are vitamin D deficient (25(OH)D level ≤ 20 ng/ml) are screened for inclusion and exclusion criteria. The screening period and recruitment period starts in May 2010 and lasts for 22 months (March 2012). Patients are randomly assigned to one of the two treatment study groups, "Vitamin D" or "Placebo", in a 1:1 ratio, stratified according to ICU and gender. Patients randomized to the "Vitamin D" group receive a loading dose of 540,000 IU of vitamin D3 orally or via feeding tube (concentration of cholecalciferol is 400 IU vitamin D3/drop of oleum arachidis; total
carrier volume is 45ml). From month 1 to 5, patients receive monthly maintenance doses of 90,000 IU cholecalciferol. Patients randomized to the “Placebo” group receive oleum arachidis in the same volume and time schedule as “Vitamin D” patients. At baseline, data on demographic and clinical characteristics of the patients are obtained. Simplified Acute Physiology Score (SAPS II), presence of comorbidities, medical history, admission diagnosis by category and relevant medication are registered. In addition, we record the need for and the number of days of hemodynamic support and mechanical ventilation. Information on insulin requirements and use of antibiotics are registered on day 0, 2 and 6. Venous blood and urinary samples are taken upon ICU admission and in the morning on days 0, 3, 7 and 28 if feasible. Analyses include serum chemistry, hematometry, markers of inflammation and parameters of calcium and vitamin D metabolism. All patients who leave the hospital are contacted by telephone on a monthly basis to check for compliance with the monthly intake of study drug (5 times) and vital status. At month 6 the patients or their caregivers are contacted for a telephone interview to retrieve information on study drug intake, tolerability and general health status of the patient. All patients are invited for a personal follow-up at the Medical University Graz, however due to social, medical or geographical reasons only a minority was able and willing to attend it. An overview of the study procedures is given in Figure 1.

497 randomisations were performed, 17 were rejected prior to the application of the study medication (5 patients were randomized twice, 12 were rejected – in 5 cases informed consent was withdrawn, in 2 cases an exclusion criteria was present and in 5 patients application of the study medication was not possible due to nausea and vomiting). 480 patients were enrolled in the study and received the study medication. After regaining consciousness 5 patients refused informed consent for further study procedures and were excluded from all analysis. Consequently, 475 patients are analysed on an intention-to-treat basis.

4. Patient Populations and Analysis Sets

Primary analysis set
The intention-to-treat population will include all patients who receive at least one dose of medication. 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. All patients included here will be analysed according to the treatment assignment during randomisation.

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. 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. All patients will be analysed according to the treatment they actually received.
5. Study Methods

5.1 Schedule

The actual timings of all assessments performed are given in Table 1. Supporting information including details of all evaluations is given in the study protocol.
### Statistical Analysis Plan

#### Table 1: Overview of time schedule

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<th>Study visit</th>
<th>Day0</th>
<th>Day2</th>
<th>Day3</th>
<th>Day6</th>
<th>Day7</th>
<th>Day28</th>
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<td>signed informed consent</td>
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<td>x</td>
<td>x</td>
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<td>recent and concomitant medication</td>
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#### Safety evaluation

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<th>blood draws*</th>
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<th>+/- 7 days</th>
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<td>laboratory assessments</td>
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<td>urinary analysis</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x (20%)</td>
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<tr>
<td>vital status</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>number of falls/fractures</td>
<td>x</td>
<td></td>
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</table>

#### Outcome variables

| laboratory assessments     | x    | x            | x          | x (20%)     |
| urinary analysis           | x    | x            | x          | x (20%)     |
| use of insulin/antibiotics | x    | x            |            |             |
| use of parenteral/enteral formulas | x | x | x | x |
| duration of mechanical ventilation | x | x | x | x |
| need for vasopressor therapy | x | x | x | x |
| upper respiratory tract infections | | | | x |
| performance status (ECOG score) | | | | x |
| quality of life (SF 12)   |      |              |            |             |
| timed up & go test| | | | x (20%) |
| hand grip strength        |      |              |            |             |
| DXA**                     |      |              |            |             |

#### Study medication

| drug dispensing | x    | x    | x    |      |
| drug accountability / compliance check | x    |      |      |    |
| drug return     | x    |      |      |      |

* Blood draws: For timing of blood draws the tolerance for still acceptable time points will be as follows: 48 hours for scheduled blood draws at days 3 and 7; 1 week for the scheduled blood draw at day 28; 1 month for the scheduled blood draw at month 6.

1 Only with personal follow-up at the Medical University of Graz (approximately 20%)

** DXA: dual energy x-ray absorptiometry

Between Day 28 and Month 6 monthly follow-up calls assess vital status and study drug intake and compliance
5.2 Primary endpoint: length of hospital stay

For the present study "the length of hospital stay" is defined as the time elapsed between application of study drug at the ICU until discharge from the hospital or a comparable institution (in hours) or death. Stays at other health care facilities like nursing homes or rehabilitation centers are not considered for hospital stay. This implies that patients who are transferred from LKH Universitätsklinikum Graz to another hospital will be carefully followed up and necessary information will be obtained either by telephone call or in written form. In cases where information from a peripheral hospital only includes "day" and not the "exact hour" of discharge, the time-point for analysis is arbitrarily set to 12 am. The time needed for transportation from one hospital to the other will be included in the calculation of the "length of hospital stay". A listing and assignment of all institutions to either "hospital or hospital-like institutions" or "health care and rehabilitation facilities" to which participating patients have been transferred will be independently classified by two members of the study team before unblinding and discussed at the blind review meeting (Appendix 1).

5.3 Secondary endpoints

5.3.1 Percentage of patients with 25(OH)D levels ≥ 30 ng/ml at day 7 in the intervention group

5.3.2 Length of ICU stay

The length of ICU stay for this study is defined as the time elapsed between application of study drug at the ICU until discharge from ICU. Following intermediate-care facilities will be considered as "ICU"-institutions: Intermediate "medical/respiratory care unit" (Department of Internal Medicine) and CK GMIÜ (Department of Surgery). The following units are not taken into account for ICU stay because they do not fulfil the criteria of an ICU (patients are only monitored). These are the following institutions: CK HEIÜ (Department of Surgery, Division of Cardiac Surgery), CK TXIMC (Department of Surgery, Division of Transplantation Surgery), NK Stroke Unit (Department of Neurology).

In analogy to the above stated remarks study patients transferred to external ICUs will be carefully followed up and necessary information will be obtained either by telephone call or in written form. In cases where information from a peripheral hospital only includes a "day" and not the "exact hour" of discharge from ICU this time-point for analysis is arbitrarily set to 12 am. The time needed for transportation from one ICU to the other will be included in the calculation of the "length of ICU stay". A listing of all ICUs to which participants of the study have been transferred to, is included (Appendix 2).

In cases where study participants have been transferred from an ICU to a hospital ward and are readmitted to an ICU within 48 hours the "length of ICU stay" will also include the time the patient spent at the hospital ward. Readmissions later than 48 hours will be handled and analysed as "hospital stay".
5.3.3 Need for and duration of mechanical ventilation during ICU stay

The need for and duration (in hours) of mechanical ventilation will be compared between the two study groups and is defined as starting with application of study medication. It comprises only the period of the endotracheal intubation and possible alternate intubation times will be added up and analysed in total. These data are merely documented for the time spent at the ICUs of the LKH Universitätsklinikum Graz and will not be considered for stays at external ICUs.

5.3.4 Need for a tracheostomy during ICU stay

The percentage of patients who received a tracheostomy during ICU stay is documented and will be compared between the two study groups.

5.3.5 Need for and duration of vasopressor therapy during ICU stay

The need for and duration (in hours) of vasopressor therapy will be compared between the two study groups and is defined as starting with application of study medication. Noradrenalin (Arterenal®, the most important vasopressor agent, will be studied. If several periods of noradrenalin requirement are necessary, they will be added up and analysed in total. Other vasopressor agents like dobutamin, levosimendan etc. will not be taken into account. These data are only documented for the time spent at the ICUs of the LKH Universitätsklinikum Graz and will not be considered for stays at external ICUs.

5.3.6 NT-proBNP levels at day 7

5.3.7 C-Reactive Protein and procalcitonin values at day 7

5.3.8 1,25(OH)D and PTH at day 7

5.3.9 TISS 28 score at day 7

5.3.10 Need for parenteral/enteral nutrition during ICU stay

The need for parenteral/enteral nutrition will be analysed as a dichotomous variable (yes/no) and will be compared between the two study groups. Moreover, the mean daily dose of vitamin D supplements (Cernevit® and Vitalipid®) routinely administered during ICU stay, related to the total time of ICU stay will be compared between the two study groups. Cernevit® contains 220 IU of cholecalciferol (vitamin D3) and Vitalipid® contains 200 IU of ergocalciferol (vitamin D2). Therefore the dose of both products is calculated with 210 IU vitamin D per ampoule.

5.3.11 Use of insulin and antibiotics at day 6

The requirement for insulin treatment (intravenous or subcutaneous) and the need for and number of antibiotics at day 6 will be compared between the two study groups.
5.4 Safety Evaluations

Safety endpoints comprise vital status (ICU and hospital mortality, 28-day mortality, 6-month mortality) as well as serum 25(OH)D and calcium levels. The vital status will be determined for all patients until month 6. In cases where the exact hour of death is unknown, 12 a.m. is used for the analysis. If there is a discrepancy between time of death reported in the hospital discharge file (openMEDOCS) and the time given in the medical record (physician report or discharge letter, time given on a chart...), the documented time given on the latter documents will be considered.

25(OH)D and serum calcium levels are measured at day 0, 3, 7 and, if available, at day 28 and month 6 in order to recognize vitamin D intoxication (25(OH)D > 150 ng/ml), hypercalcemia (total calcium > 2.65 mmol/l or ionized calcium >1.35 mmol/l) and/or hypercalciuria (urinary calcium-creatinin-ratio > 0.60).

Blood sample for laboratory evaluations are collected at day 0, 3, 7, 28 and at month 6 in case of a personal follow-up. Laboratory data recorded will be analysed quantitatively and qualitatively. Qualitative analysis will be done comparing the laboratory data to their reference ranges. Values outside the reference range will be tabulated.

After the study by Sanders et al. demonstrated a higher risk of falls and fractures in the annual high-dose vitamin D group in elderly women in May 2010, we decided to add the number of self-reported falls and fractures in the 6-month follow up as additional safety endpoint at 6 months.

5.5 Missing Data

All patients should complete all the required assessments at each visit. The eCRF database includes all data items as they are recorded. All available data will be used in the analyses and data summaries. There will be no imputation of any missing data. To ensure that the original data is always available any derived variables will be added as additional variables.

5.6 Predefined Subgroup Analysis

As far as the primary and secondary endpoints are concerned the vitamin D intervention group will be analysed separately for study patients with baseline 25(OH)D >12 and ≤12 ng/ml. Because patients with 25(OH)D ≤12 ng/ml show a severe vitamin D deficiency which is often associated with osteomalacia, a high-dose supplementation may achieve a more pronounced statistical significance in the investigated endpoints.
6. Statistical Methods

All clinical and safety data collected in the study will be analysed and reported with SAS v9.2 procedures in a Windows XP environment.

6.1 Analysis of primary endpoint

The primary hypothesis:

H0: There will be no clinically important difference in the length of hospital stay in participants treated with vitamin D vs. placebo.

The primary analysis for comparing length of hospital stay between the two groups will be made using the Mann–Whitney U-test. Sensitivity analyses will consider time to hospital discharge as survival endpoint with death as competing risk.

6.2 Analysis of secondary endpoints

For secondary endpoints, differences between the two groups will be evaluated with the t test or Mann–Whitney U-test for continuous variables as appropriate. Laboratory parameters having a skewed distribution will be log-transformed. For categorical variables the chi-square test or Fisher Exact test will be used. Survival endpoints will be displayed in Kaplan-Meier plots and compared by the log rank test. Furthermore, linear, logistic regression or Cox regression models, as appropriate, will be applied to adjust for age, acute disease severity (SAPS II) and comorbidity index (Charlson Index).

6.3 Demographic and Baseline Characteristics

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

6.4 Sample Size Estimation

The sample size was calculated to detect a difference in mean length of hospital stay of 2 days with 80% power and a significance level of 5%. Using the Mann-Whitney U-Test for sample size calculation, a group size of 234 is needed to show a reduction of 2 days (equivalent to 14%) of the primary study outcome based on a mean hospital
stay of 14 days and standard deviation of 7 days for the control group. To consider drop-outs of the study, a sample of 480 patients (240 per arm) was considered appropriate.

6.5 Presentation

All 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 summarised using frequencies and percentages.

Each summary table will be supported by individual subject data listings.
Figure 1: Overview of VITdAL@ICU study procedures

**Screening for inclusion/exclusion criteria**
- Vitamin D deficiency (25(OH)D<20ng/ml)
- Patient > 18yrs
- Expected ICU stay > 48h

**Exclusion criteria**
- unable to take study medication;
- DNR/imminent death; hypercalcemia;
- nephrolithiasis, tuberculosis or sarcoidosis; pregnancy/lactation; other ongoing trial; consent refusal

**Informed Consent**
- Inclusion
- Randomization stratified by ICU and gender

**Day 0: Inclusion**
- Assigned to vitamin D (540,000 IU cholecalciferol)
- Blood and urine draw

**Day 2-7**
- Daily CRF (i.e. antibiotics, insulin...)
- Blood/urine draws on day 3 and 7

**Day 8-28**
- Distribution of study medication for home use
- Blood/urine draw at day 28 if feasible

**Month 2-6**
- 90,000 IU cholecalciferol or corresponding placebo monthly
- Monthly telephone calls to check for study medication intake and vital status
- 6 month-visit and 2 year visit in two variants
  1. Follow-up by telephone
  2. Visit at the clinic including blood/urine sample, clinical examination and dual X-ray absorptiometry
VIItdAL@ICU - Study

Listing of external hospitals or hospital-like institutions

- LKH Graz West
- KH der Elisabethinen Graz
- KH der Barmherzigen Brüder Graz Eggenberg
- KH der Barmherzigen Brüder Graz Marschallgasse
- Privatklinik Kastamnienhof Graz
- LKH Hartberg
- LKH Hörgas-Enzenbach
- LKH Deutschlandsberg
- LKH Voitsberg
- LKH Bad Radkersburg
- LKH Bruck an der Mur
- LKH Weiz
- LKH Wagna
- LKH Mürzzuschlag-Mariazell
- LKH Judenburg-Knittelfeld
- LKH Rottenmann
- Krankenhausverbund Feldbach-Fürstenfeld
- Marienkrankenhaus Vorau
- LKH Güssing
- LKH Oberwart
- KH Gmunden
- Landesklinikum Wiener Neustadt
- KH Rudolfstiftung Wien
- KH Rosenhügel
- LKH Horn
- KH Spital an der Drau
- LKH Villach
- LKH Klagenfurt
- KH Olmütz (Tschechien)

1 This listing comprises all hospitals or hospital-like institutions to which participants of the VIItdAL@ICU study have been transferred to (transfer to general ward).

VIItdAL@ICU – Appendix 1
Listing of external health care and rehabilitation facilities

Rehabilitation facilities

- Privatklinik Laßnitzhöhe
- Albert-Schweitzer-Klinik (geriatric hospital)
- Kurklinik Maria Theresia Bad Radkersburg
- Landesnervenklinik Sigmund Freud Graz (neurologic rehabilitation ward)
- LKH Hörgas-Enzenbach (acute geriatric remobilisation ward)
- KH Judendorf-Straßengel (neurologic rehabilitation ward)
- LKH Judenburg-Knittelfeld (neurologic rehabilitation ward)
- LKH Rottenmann (remobilisation ward)
- LKH Klagenfurt (neurologic rehabilitation ward)
- Rehabilitationsklinik Tobelbad
- SKA Rehabilitationszentrum Bad Tatzmannsdorf
- SKA Rehabilitationszentrum St. Radegund
- SKA Rehabilitationszentrum Bad Ischl
- SKA Rehabilitationszentrum Bad Schallerbach

Health care facilities

- Pflegeheim Kalsdorf
- Pflegeheim Wagna
- Pflegeheim Birkfeld
- Pflegeheim Stegersbach
- Pflegeheim Haus der Barmherzigkeit Graz
- Pflegezentrum Grazerfeld Süd
- Caritasheim St. Peter am Ottersbach
- Geriatriezentrum Am Wienerwald
- Humanitasgruppe Unterpremstätten
- Kurhaus Bad Gleichenberg

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2 This listing comprises all health care and rehabilitation facilities to which participants of the VITdAL@ICU study have been transferred to.
VITdAL@ICU - Study

Listing of external hospitals – transfer to intensive care unit/s

- LKH Graz West
- KH der Elisabethinen Graz
- KH der Barmherzigen Brüder Graz Marschallgasse
- LKH Hartberg
- LKH Hörgas-Enzenbach
- LKH Deutschlandsberg
- LKH Voitsberg
- LKH Bruck an der Mur
- LKH Wagna
- LKH Leoben-Eisenerz
- LKH Judenburg-Knittelfeld
- Krankenhausverbund Feldbach-Fürstenfeld
- LKH Güssing
- LKH Oberwart
- KH Gmunden
- Landesklinikum Wiener Neustadt
- AKH Wien Neurochirurgie

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1 This listing comprises all hospitals to which participants of the VITdAL@ICU study have been transferred to (transfer to ICUs).

VITdAL@ICU - Appendix 2