PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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)
AUTHORS	Amrein, Karin; Parekh, Dhruv; Westphal, Sabine; Preiser, Jean; Berghold, Andrea; Riedl, Regina; Eller, Philipp; Schellongowski, Peter; Thickett, David; Meybohm, Patrick

VERSION 1 – REVIEW

REVIEWER	Robert Scragg University of Auckland, New Zealand.
REVIEW RETURNED	03-May-2019

GENERAL COMMENTS	The authors have submitted a manuscript reporting an abstracted version of the protocol for a large multicentre randomized controlled trial to determine if high-doses of vitamin D supplements reduce mortality in ICU patients with severe vitamin D deficiency (25-hydroxyvitamin D <12 ng/ml). The study is well-justified as it aims to confirm unexpected results from a previous study by the lead author which surprisingly showed reduced mortality in ICU patients with similar very low levels of 25-hydroxyvitamin D. The trial is very important, because if the earlier study is replicated, the widespread use of vitamin D as an adjunct therapy for these patients most likely will become widespread. Overall, the study design is excellent. The dose of vitamin D is similar to that of the first study (aside from changing follow-up doses from monthly to their similar daily equivalent). The researchers have set in place appropriate monitoring of safety, important because of the high bolus dose, by recording relevant adverse events such as falls, fractures and kidney stones. My only query here is why there are no follow-up measures of calcium and 25-hydroxyvitamin D after Day 5. No doubt this is because it will be difficult to collect blood samples after this time-point. But if this is possible in a subgroup, it will greatly help the researchers interpret their findings, particularly if there is no effect of vitamin D on the outcomes or if adverse events are found to be increased in the vitamin D arm. Careful attention has been given to the sample size. Thus, a very large trial is planned (aim is 2400 participants), based on a conservatively estimated effect of vitamin D on mortality (risk ratio of 0.80).

	My main comments about the manuscript relate to lack of detail about the study endpoints and methods which one normally would expect to see in a publication of this type. This comment particularly relates to the measures reported on page 7 of the manuscript, specifically: • Sequential Organ Function Assessment Score • Katz Activities of Daily life • EQ-5D-5L • WHO-DAS 2.0 Also, detail should be added about how this information will be collected, particularly the mortality at Day 28 (the primary outcome) and other outcomes at Day 90. Readers will not want to go to the study protocol to search out this information. Other specific comments are: 1. Page 3 of the manuscript, line 47-48: reference 11 from the VITAL study was a study of participants selected from the community, not of ICU patients. Please correct this. 2. What is the origin of the study acronym? Readers like to know this. 3. Page 11 of the manuscript, second paragraph. Please provide a reference for the VIOLET trial so that interested readers can locate it. 4. The figure of the CONSORT flow diagram has a number of abbreviations which should be footnoted so that readers don't have to read through the text of the manuscript to find out what they mean.

REVIEWER	Ling Li Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China.
REVIEW RETURNED	13-Jun-2019

GENERAL COMMENTS	I read this manuscript form Amrein K et al, the author's design is impeccable, the topic is interesting, however, there are no a clear conclusion, just a protocol before 5 years, I do not know whether the study is suitable for BMJ OPEN. In addition, their main research results were published in BMC Endocr disord. 2012 Nov 7;12:27; JAMA. October 15, 2014; at least. I don't think this study is innovative and significative, although the protocol is OK.

REVIEWER	Yan-Peng Zhang Zhejiang university China
REVIEW RETURNED	14-Jul-2019

GENERAL COMMENTS	Lower hospital mortality was observed in the severe vitamin D deficiency subgroup (25(OH)D ≤12 ng/ml) as a secondary endpoint in the VITdAL-ICU randomised, double-blind, placebo-controlled, single-centre trial. As the author mention, this VITDALIZE study provided significant differences between vitamin D and placebo group concerning the 28-day mortality. COMMENTS : 1. Description of vitamin D in the first paragraph of introduction was duplicate with another paragraph in discussion. For example, "Vitamin D is a precursor of a steroid hormone with a specific nuclear receptor …and seems to predispose to a variety of respiratory, immune, infectious, neurologic and cardiovascular

	diseases" in the introduction was duplicate with "Vitamin D has much broader, pleiotropic effects that extend well beyond the musculoskeletal system. 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" in the discussion. Please revise. 2. Background-much is written in this section. This section was unduly long and part of the content should be placed in the discussion. 3. Nutrition. 2017 Jun;38:102-108. doi: 10.1016/j.nut.2017.02.002.This study found a dose-related increase in plasma free-25(OH)D levels, which was associated with increasing circulating mRNA expression of hCAP18 over time. Larger studies appear warranted to determine the impact of high-dose vitamin D3 administration on endogenous AMPs. I am just wondering whether high-dose vitamin D3 administration has some side effects or impacts the release of endogenous factors? Add some thinking into the discussion part.

VERSION 1 – AUTHOR RESPONSE

Reviewers' Comments to Author:

Reviewer: 1
Reviewer Name: Robert Scragg
Institution and Country: University of Auckland, New Zealand.
Please state any competing interests or state 'None declared': None declared

The authors have submitted a manuscript reporting an abstracted version of the protocol for a large multicentre randomized controlled trial to determine if high-doses of vitamin D supplements reduce mortality in ICU patients with severe vitamin D deficiency (25-hydroxyvitamin D <12 ng/ml). The study is well-justified as it aims to confirm unexpected results from a previous study by the lead author which surprisingly showed reduced mortality in ICU patients with similar very low levels of 25-hydroxyvitamin D. The trial is very important, because if the earlier study is replicated, the widespread use of vitamin D as an adjunct therapy for these patients most likely will become widespread.
Overall, the study design is excellent. The dose of vitamin D is similar to that of the first study (aside from changing follow-up doses from monthly to their similar daily equivalent). The researchers have set in place appropriate monitoring of safety, important because of the high bolus dose, by recording relevant adverse events such as falls, fractures and kidney stones.

My only query here is why there are no follow-up measures of calcium and 25-hydroxyvitamin D after Day 5. No doubt this is because it will be difficult to collect blood samples after this time-point. But if this is possible in a subgroup, it will greatly help the researchers interpret their findings, particularly if there is no effect of vitamin D on the outcomes or if adverse events are found to be increased in the vitamin D arm.
This is a very important point and we like to thank the reviewer for this remark. Based on the previous intervention studies that we performed with a similar dosing regimen, the peak vitamin D Level is seen around day 3. Patients are followed with routine calcium levels and hypercalcemia is a possibly Vitamin D related adverse event that should be reported.

Careful attention has been given to the sample size. Thus, a very large trial is planned (aim is 2400 participants), based on a conservatively estimated effect of vitamin D on mortality (risk ratio of 0.80).

My main comments about the manuscript relate to lack of detail about the study endpoints and methods which one normally would expect to see in a publication of this type. This comment particularly relates to the measures reported on page 7 of the manuscript, specifically:

- Sequential Organ Function Assessment Score
- Katz Activities of Daily life
- EQ-5D-5L
- WHO-DAS 2.0

Also, detail should be added about how this information will be collected, particularly the mortality at Day 28 (the primary outcome) and other outcomes at Day 90. Readers will not want to go to the study protocol to search out this information.

Many thanks for this very important point. We have revised the section and added additional information.

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 (PaO2/FiO2), 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.

Other specific comments are:

1. Page 3 of the manuscript, line 47-48: reference 11 from the VITAL study was a study of participants selected from the community, not of ICU patients. Please correct this.

Thank you very much for this remark. The reference 11 has been removed.

2. What is the origin of the study acronym? Readers like to know this.
VITDALIZE is indeed a chosen name (a wordplay, hoping to “vitalize” patients with vitamin D), not an acronym.

3. Page 11 of the manuscript, second paragraph. Please provide a reference for the VIOLET trial so that interested readers can locate it.
We thank the reviewer for this helpful comment. Unfortunately, results have not yet been published, but the study identification number of clinicaltrials.gov has been inserted.
“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 results have not yet been published.”

4. The figure of the CONSORT flow diagram has a number of abbreviations which should be footnoted so that readers don’t have to read through the text of the manuscript to find out what they mean.
Thank you very much for this valuable comment. A list of abbreviations has been added to the Figure.
“Abbreviations: ICU: Intensive Care Unit; IU: International Unit; MCT: Median Chain Triglycerides; SOFA: Sequential Organ Failure Assessment”

Reviewer: 2
Reviewer Name: Ling Li
Institution and Country: Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China.
Please state any competing interests or state ‘None declared’: None.

I read this manuscript form Amrein K et al, the author's design is impeccable, the topic is interesting, however, there are no a clear conclusion, just a protocol before 5 years, I do not know whether the study is suitable for BMJ OPEN. In addition, their main research results were published in BMC Endocr disord. 2012 Nov 7;12:27; JAMA. October 15, 2014; at least. I don't think this study is innovative and significative, although the protocol is OK.

We thank the reviewer for this revision. As mentioned in the manuscript, 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, hazard ratio (HR) 0.52 (0.30-0.89), number needed to treat = 6). As this was only a secondary endpoint in the predefined subgroup with severe vitamin D deficiency, this finding is hypothesis generating and needs a confirmatory study. High-dose vitamin D3 in a population of severely vitamin D deficient critically ill patients is a promising and inexpensive intervention. Therefore we want to confirm these findings in the multicenter study VITDALIZE.
Research results of the VITDALIZE study are not yet available and have not yet been published for this reason. The publications mentioned (BMC Endocr disord. 2012 Nov 7;12:27; JAMA. October 15, 2014) refer exclusively to the VITdAL-ICU study, THE PREVIOUS STUDY.

Reviewer: 3
Reviewer Name: Yan-Peng Zhang
Institution and Country: Zhejiang university, China Please state any competing interests or state ‘None declared’: None declared

Lower hospital mortality was observed in the severe vitamin D deficiency subgroup (25(OH)D ≤12 ng/ml) as a secondary endpoint in the VITdAL-ICU randomised, double-blind, placebo-controlled, single-centre trial. As the author mention, this VITDALIZE study provided significant differences between vitamin D and placebo group concerning the 28-day mortality.

COMMENTS :

1. Description of vitamin D in the first paragraph of introduction was duplicate with another paragraph in discussion. For example, “Vitamin D is a precursor of a steroid hormone with a specific nuclear receptor …and seems to predispose to a variety of respiratory, immune, infectious, neurologic and cardiovascular diseases” in the introduction was duplicate with “Vitamin D has much broader, pleiotropic effects that extend well beyond the musculoskeletal system. 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” in the discussion. Please revise.
Thank you very much for carefully reading the manuscript. The duplicates were removed from the “Discussion” section.

2. Background-much is written in this section. This section was unduly long and part of the content should be placed in the discussion.
We thank the reviewer for this very valuable comment. We transferred important information to the “Discussion” section.

3. Nutrition. 2017 Jun;38:102-108. doi: 10.1016/j.nut.2017.02.002.This study found a dose-related increase in plasma free-25(OH)D levels, which was associated with increasing circulating mRNA expression of hCAP18 over time. Larger studies appear warranted to determine the impact of high-dose vitamin D3 administration on endogenous AMPs. I am just wondering whether high-dose vitamin D3 administration has some side effects or impacts the release of endogenous factors?
Add some thinking into the discussion part.
This is a very interesting study and we would like to thank the reviewer for this comment. We added information about this trial into the “Discussion” section.
“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 that may have beneficial effects on critical illness and inflammatory outcomes.”

VERSION 2 – REVIEW

REVIEWER	Robert Scragg University of Auckland New Zealand
REVIEW RETURNED	01-Sep-2019

GENERAL COMMENTS	The authors have responded satisfactorily to all my initial queries and concerns.

REVIEWER	Yan-Peng Zhang China
REVIEW RETURNED	25-Aug-2019

GENERAL COMMENTS	the manuscript meets the criteria for publication