Trials

STUDY PROTOCOL

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# Comparison of two doses of vitamin D3 in critically ill patients undergoing continuous renal replacement therapy (NephroD): study protocol for a single-blinded, multicenter, parallel group randomized controlled trial

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## Abstract

**Background** ICU patients are particularly susceptible to vitamin D3 deficiencies. This can be due to the severity of their underlying disease, the type of treatment they are on, and malnutrition before and inadequate nutrition during the hospitalization preceding ICU admission as well as advanced age. Literature provides no guidance on how to supplement vitamin D3 in severely deficient patients who are undergoing continuous renal replacement therapy (CRRT). Most serum 25(OH)D3 is bound with vitamin D binding protein in a complex whose molecular weight is 10 kDa. This means it can be removed during CRRT via convection mechanism. Critically ill patients undergoing CRRT can therefore be particularly prone to develop severe vitamin D3 deficiency.

**Methods** As the trial design, a randomized controlled, single blinded, multicenter, parallel group approach was chosen to compare a single administration of 750,000 IU of vitamin D3 via the enteral or oral route in ICU patients with severe vitamin D3 deficiency (measured serum 25(OH)D3 levels ≤ 12.5 ng/ml) undergoing CRRT with a single administration of 500,000 IU of vitamin D3. The trial will be performed in up to five university hospitals in Poland. The primary outcome is the percentage of patients that achieved serum 25(OH)D3 levels ≥ 30 ng/ml on days 3 and 7 following vitamin D3 administration. Assuming a drop-out rate of approximately 10%, the number of recruited patients should be 138.

**Discussion** Considering the potential pathophysiological mechanisms underlying hypovitaminosis D in critically ill patients under CRRT, it seems conceivable that these patients will require greater supplementation doses to correct severe deficiency. The study is meant to help answer the question whether increasing the supplementation dose by 50% will ensure a more effective replenishment of vitamin D3 in critically ill patients undergoing CRRT.

**Trial registration** ClinicalTrials.gov Identifier: NCT05657678, registered: December 12 2022, https://clinicaltrials.gov/study/NCT05657678?cond=NCT05657678&rank=1.

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## Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

| | |
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## Introduction

### Background and rationale {6a}

The main function of vitamin D3 in the human body is its influence on extracellular calcium metabolism. In addition to this classic role, vitamin D3 also has a unique pleiotropic function [1–7]. This pleiotropy, which refers to the parallel action of one factor on a number of secondary biomechanisms, may lead to vitamin D3 having the following effects:

- Inhibition of cancer cell proliferation—vitamin D3 has been shown to play a role in inhibiting the development of many neoplasms
- Modulation of the immune system, which may prevent autoimmune diseases and severe infections
- Effect on the cardiovascular system—vitamin D3 deficiency has been shown to be associated with increased incidence of hypertension and ischemic heart disease

Vitamin D3 deficiency is relatively common in the healthy population. This is because the endogenous formation of vitamin D3 depends on age, season, latitude, and skin color. Vitamin D3 insufficiency is a predisposing factor for many respiratory, cardiovascular, neural, and immune diseases as well as severe infections. In the blood, vitamin D3 is mostly bound to the carrier vitamin D binding protein (DBP) and to a lesser extent to albumin. In the liver, vitamin D3 undergoes hydroxylation to the active prohormone 25–hydroxyvitamin D3 (25(OH) D3). The serum concentration of 25(OH)D3 is considered to reflect the body's total store of vitamin D3. Due to its stability and relatively long half-life (2–3 weeks), this form of vitamin D3 is used as a marker of vitamin D3 reserves. A 25(OH)D3 concentration of $<30$ ng/ml is considered low, $<20$ ng/ml is considered deficient, and $\leq 12$ ng/ml is considered a severe deficiency of vitamin D3 [1–7].

Intensive care unit (ICU) patients are particularly susceptible to vitamin D3 deficiencies. This depends on the severity of the underlying disease, the type of treatment patients are on, and malnutrition before and inadequate nutrition during the hospitalization preceding ICU admission as well as advanced age. It has also been established that serum levels of 25(OH)D3 tend to decrease systematically during ICU treatment [8]. Interventions in ICU such as fluid resuscitation or extracorporeal therapy can cause vitamin D3 deficiencies, while acute liver

failure or acute kidney injury impair the vitamin's metabolic pathway. The incidence of vitamin D3 deficiency can reach up to 90%, and even 30% of ICU patients may have vitamin D3 undetectable serum levels. It is impossible to replenish vitamin D3 levels in critically ill patients with traditional enteral and parenteral nutrition treatment regimens because nutritional products contain too little of the vitamin [8].

Vitamin D3 deficiency has been associated with increased all-cause ICU mortality. However, the assessment of serum 25(OH)D3 levels is not routine practice in ICUs. In view of the prevalence of vitamin D3 deficiencies in ICU patients, rapid replenishment of this deficiency with an increased supplementation dose should be considered in high-risk patients with extremely low serum 25(OH)D3 levels as a potential means to improve prognosis in this patient population [9–22].

In the VITdAL@ICU study, which was the first large multicenter supplementation study, ICU patients were administered a single enteral dose of 540,000 IU of vitamin D3 to quickly correct the deficiency. The study did not reveal any differences in hospital mortality or mortality at day 180 compared with the control group. However, a trend towards reduced hospital mortality was shown in severely vitamin D3 deficient patients (serum levels $\leq$ 12 ng/ml) ($n=200$, 28.6 vs 46.1%, $p=0.01$, HR 0.56; 95% CI, 0.35–0.90) [23]. The results of the VITdAL@ICU study suggest that critically ill patients with a severe deficiency of vitamin D3 might benefit the most from early high dose supplementation. The multicenter, randomized VIOLET study, which investigated the potential benefits of the early administration of high-dose vitamin D3 in ICU patients, did not show any beneficial effects. However, the study assumed a supplementation threshold of 20 ng/ml, which is debatable given that the VITdAL@ICU study suggested potential benefits to be limited to severely deficient patients [24]. Another large, multicenter, randomized study (VITDALIZE) is currently ongoing, investigating the connection between early enteral high-dose supplementation and mortality rates in critically ill patients with severe vitamin D3 deficiency (serum levels equal to or lower than 12 ng/ml). The results of the study are expected to emerge over the next few years [25].

Vitamin D3 supplementation in critically ill patients is markedly different from supplementation in the general population. Oral supplementation doses in healthy people usually amount to 2000–4000 IU daily. Correcting a pre-existing deficiency with this dosing regimen can take many weeks. The 2019 recommendations of the European Society for Clinical Nutrition and Metabolism (ESPEN) regarding nutritional treatment in intensive care suggested one-off enteral supplementation with 500,000 IU of vitamin D3 in patients diagnosed with severe deficiency (serum concentration of vitamin D3 $\leq$ 12.5 ng/ml). The administration of vitamin D3 was recommended within 7 days following ICU admission [26]. This recommendation did not distinguish between intensive care patients who undergo or did not undergo continuous renal replacement therapy (CRRT). The above recommendations were updated in 2023. Currently, it is recommended that serum vitamin D3 levels should be measured in all patients admitted to the ICU. The supply of a specific dose of vitamin D3 and the timing of administration are no longer recommended. The paper emphasizes that further clinical studies are needed on how to dose vitamin D3 in critically ill patients treated in ICUs [27].

Approximately 35–65% of all ICU-treated patients suffer from acute kidney injury. Among septic patients with concomitant acute kidney injury, mortality can reach up to 70%. Renal replacement therapy is a common type of treatment in ICU patients with symptoms of acute kidney injury (apart from effective treatment of the underlying disease). Two types of renal replacement therapy are usually used: intermittent hemodialysis or continuous renal replacement therapy (CRRT). The latter is used particularly in critically ill patients with hemodynamic instability.

The literature provides no guidance on how to supplement vitamin D3 in deficient patients who are undergoing renal replacement therapy in the ICU. This issue mostly revolves around CRRT, which seems to be associated with a significantly greater risk of increased vitamin D3 depletion. Most serum 25(OH)D3 is bound to DBP in a complex whose molecular weight is approximately 10 kDa. This means that it can be removed during CRRT via a convection mechanism. In addition, 25(OH)D3 may be absorbed by certain elements of the extracorporeal circuit, and the increased volume of distribution related to CRRT can lead to a relative decrease in vitamin D3 plasma levels. In addition, in the event of hypoalbuminemia, an intensified loss of vitamin D3 via diffusion (increased free 25(OH)D3 fraction in the blood) can be expected during CRRT. Critically ill patients undergoing CRRT can therefore be particularly prone to developing severe vitamin D3 deficiency [28]. Considering the potential pathophysiological mechanisms underlying hypovitaminosis D in critically ill patients undergoing CRRT, it seems conceivable that these patients will require greater supplementation doses to correct this severe deficiency.

The NephroD study aims to examine whether increasing the supplementation dose by 50% can ensure a more effective replenishment of vitamin D3 in critically ill patients undergoing CRRT.

#### Objectives {7}

The primary objective is to compare the effects of two different supplementation doses of vitamin D3—500,000 IU or 750,000 IU administered as one enteral dose (via a nasogastric tube or orally)—on the serum levels of vitamin D3 in ICU patients undergoing CRRT and diagnosed with severe vitamin D3 deficiency.

Secondary objectives:

- To compare the effects of two different supplementation doses of vitamin D3 on mortality in ICU patients undergoing CRRT
- To compare the effects of two different supplementation doses of vitamin D3 on the duration of ICU treatment in patients undergoing CRRT
- To compare the effects of two different supplementation doses of vitamin D3 on Sequential Organ Failure Assessment (SOFA) scores in ICU patients undergoing CRRT
- To compare the effects of two different supplementation doses of vitamin D3 on the duration of catecholamine administration in ICU patients undergoing CRRT
- To evaluate the relationship between the length of CRRT use in hours from the time of drug administration to the beginning of visit 4 and serum vitamin D3 levels in both study arms
- To assess the relationship between total gastric residual volume (GRV) in milliliters from the time of vitamin D3 administration to the date of the beginning of visit 3, and serum vitamin D3 levels in both study arms

The objective regarding safety was to evaluate and compare the toxicity of two different supplementation doses of vitamin D3 in ICU patients undergoing CRRT.

#### Trial design {8}

NephroD is a phase IV, randomized controlled, single blinded, multicenter, parallel group, superiority trial. The study will take place at ICUs in Poland. Patients will be randomly assigned at a ratio of 1:1 to two study arms:

1. Interventional arm—a single administration of 750,000 IU of vitamin D3 via the enteral route (through a gastric tube or orally) in ICU patients with severe vitamin D3 deficiency (measured serum 25(OH)D3 levels ≤ 12.5 ng/ml) undergoing CRRT with continuous veno-venous hemodiafiltration (CVVHDF) or continuous veno-venous hemofiltration (CVVHF)
2. Control arm (standard treatment)—a single administration of 500,000 IU of vitamin D3 via the enteral route (through a gastric tube or orally) in ICU patients with severe vitamin D3 deficiency (measured serum 25(OH)D3 levels ≤ 12.5 ng/ml) undergoing CRRT with CVVHDF or CVVHF

## Methods: participants, interventions, and outcomes

#### Study setting {9}

The NephroD trial will be performed in up to five university hospitals in Poland. Currently, four centers are active and recruiting participants namely (1) Department of Anesthesiology, Intensive Care and Regional ECMO Center, Institute of Medical Sciences, University of Opole, (2) Department of Anesthesiology and Intensive Care, Faculty of Medical Sciences in Zabrze, (3) 2nd Department of Anesthesiology and Critical Care, Medical University of Lublin, and (4) Jagiellonian University Medical College, Centre for Intensive Care and Perioperative Medicine.

Patients are considered for inclusion if they meet the criteria as defined below.

#### Eligibility criteria {10}

Clinical study inclusion criteria:

1. Presence of at least one of the following indications for initiation of CRRT with CVVHDF or CVVHF:
   - Replacement of kidney function in acute kidney injury
   - Hyperkalemia
   - Metabolic acidosis
   - Pulmonary edema
   - Uremic complications (bleeding disorder, pericarditis)
   - Hypervolemia
   - Support of renal function (volume control, regulation of acid–base and electrolyte status)
2. Sequential Organ Failure Assessment (SOFA) score of minimum 5 points at enrolment
3. Age > 18 years
4. Serum 25(OH)D3 levels ≤ 12.5 ng/ml as measured by the local laboratory of a participating hospital
5. Properly managed enteral nutrition (via a nasogastric tube or orally) regardless of dosing

Clinical study exclusion criteria:

1. Acute or advanced chronic liver failure (estimated on the basis of the clinical picture and biochemical markers: serum bilirubin, serum AST and ALT, high serum AST/ALT ratio, hypoglycemia, INR)
2. Hypercalcemia (total calcium concentration > 11 mg/dl or > 2.7 mmol/l)
3. Any parathyroid disorder
4. End stage renal disease
5. Patients undergoing plasmapheresis, extracorporeal membrane oxygenation (ECMO), extracorporeal carbon dioxide removal (ECCO2R)
6. Patients who, in the opinion of the investigator, are not expected to survive 72 h after enrolment
7. A history of nephrolithiasis or de novo nephrolithiasis
8. Patients qualified for a protocol for the avoidance of futile therapy
9. Pregnancy
10. Sarcoidosis
11. Risk of impaired intestinal absorption caused by the critical illness, associated with at least one of the following: impaired peristalsis and delayed gastric emptying, constipation, diarrhea, shock-induced intestinal hypoperfusion, hyperhydration with resulting intestinal edema following fluid resuscitation, intestinal flora disorders.

#### Who will take informed consent? {26a}

The investigator will be responsible for obtaining informed consent for study participation directly from conscious patients and documenting it on a dedicated paper form. For unconscious patients, consent for study participation must be obtained from the legal guardian or—if no legal guardian has been appointed—on the basis of consent obtained from a guardianship court. In the latter case, informed consent must be additionally sought directly to the patient as soon as he/she regains the ability to express consent for participation in the study. The investigator must inform the eligible subject of the scientific nature of the study, its objectives, and planned procedures. The investigator is obliged to answer all questions regarding the study as well as provide information on study participation, methods of personal data collection and processing, and insurance terms and conditions. The investigator should make the subject aware of the subject's study-related obligations, the associated risks and benefits, the option to withdraw from the study and the foreseeable circumstances or reasons why participation might be terminated, the duration of the study, and the approximate number of people to be enrolled. In addition, the investigator responsible for the informed consent process must ensure his/her contact data are provided on the informed consent form, along with the contact data of the principal investigator (if consent is obtained by a sub-investigator). Once a patient has expressed verbal consent to participate in the study, the investigator will assign him/her a unique study subject number, after which both the patient and the investigator will sign two copies of the informed consent form. An original copy of the consent will be given to the patient.

#### Additional consent provisions for collection and use of participant data and biological specimens {26b}

N/A. No ancillary or sub-studies are planned.

## Interventions

#### Explanation for the choice of comparators {6b}

The intervention constitutes one administration of 750,000 IU of vitamin D3 via the enteral route—into the gastric tube or orally. This dose has been established empirically as a compromise between potentially effective supplementation and the risk of overdose and toxicity. The interventional arm will be compared to standard therapy, i.e., one administration of 500,000 IU of vitamin D3 via the enteral route. The standard therapy is based on Recommendations 36 and 37 of the European Society for Clinical Nutrition and Metabolism 2019 guidelines set forth in a publication on clinical nutrition in intensive care [26]:

- In critically ill patients with measured serum levels of 25(OH)D3 < 12.5 ng/ml, supplementation should be considered (2019 ESPEN Recommendation 36)
- In critically ill patients with measured serum levels of 25(OH)D3 < 12.5 ng/ml, a 500,000 UI dose of vitamin D3 as a single administration should be considered within the first 7 days of treatment at the intensive care unit (2019 ESPEN Recommendation 37)

The NephroD study assumes a supplementation threshold of 12.5 ng/ml (25(OH)D3 concentration in serum), which is the same as that adopted in the 2019 ESPEN guidelines. According to the current 2023 ESPEN guidelines, the supply of a specific dose of vitamin D3 and the timing of administration are no longer recommended. However, current ESPEN recommendations were introduced after the start of the NephroD study. ESPEN also emphasizes that further clinical studies are needed on what doses of vitamin D3 in critically ill patients treated in intensive care units [27].

#### Intervention description {11a}

Patients will be randomly assigned at a ratio of 1:1 to two study arms:

1. Interventional arm—a single administration of 750,000 IU of vitamin D3 via the enteral route (through a gastric tube or orally) in ICU patients with severe vitamin D3 deficiency (measured serum 25(OH)D3 levels ≤ 12.5 ng/ml) undergoing CRRT with CVVHDF or CVVHF
2. Control arm (standard treatment)—a single administration of 500,000 IU of vitamin D3 via the enteral route (through a gastric tube or orally) in ICU patients with severe vitamin D3 deficiency (measured serum 25(OH)D3 levels ≤ 12.5 ng/ml) undergoing CRRT with CVVHDF or CVVHF

The study envisages the administration of a commercially available vitamin D3 medicinal product (cholecalciferol) (vitamin D3, 15,000 IU/ml, oral drops 10 ml (1 pack), 1 ml = 30 drops = 15,000 IU; 1 drop = 500 IU) (Devikap, Polpharma SA, Poland). The drug will be ordered by the investigator, i.e., the ICU physician who will be taking part in the NephroD study. The drug will then be prepared and administered by a nurse (also a member of the study team at the ICU participating in the NephroD study) and supervised by the investigator. Vitamin D3 will be administered via the enteral or oral route.

#### Criteria for discontinuing or modifying allocated interventions {11b}

Upon recruitment of 50% of the target patient population, a safety analysis will be conducted by an independent committee appointed by the Institute of Medical Sciences of the University of Opole. The analysis will focus on potential toxicities associated with high-dose vitamin D3 supplementation. The committee responsible for the safety analysis will be empowered to terminate the study at this stage.

The collection of data and follow-up of the study subjects will continue until the end of the study, unless any of the following conditions for early termination apply:

- Patient death
- Termination by choice (withdrawal of consent to participate in the study)
- Loss to follow-up
- Termination of the study or site closure by decision of the sponsor or regulatory authorities.

The investigator will record all data collected prior to patient withdrawal on the electronic case report form (eCRF), along with the reason(s) for early withdrawal, date, and adverse events. Patients who decide to withdraw their consent for continued participation in the study will no longer be contacted for the purposes of data collection. Patients who terminate their participation early will not be replaced with new patients.

#### Strategies to improve adherence to interventions {11c}

All study sites' personnel will be extensively trained in the specific protocol procedures by study monitors before site activation. In addition, cyclical online meetings with site investigators will take place with the primary investigator in order to improve the screening process and augment adherence to study interventions. During the study, the sponsor or a designee will regularly visit the sites to ensure compliance with the protocol and international conference on the harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) and good clinical practice (GCP) guidelines. The sponsor representative will have access to source documentation in order to verify the accuracy of data entered in the eCRF. The study team will provide the sponsor's representative and relevant authorities with direct access to source documentation for monitoring purposes. The study site can be inspected by auditors or representatives of the sponsor, and/or can be controlled by regulatory authorities. The investigator and his/her study team will be available during monitoring visits and audits/inspections and will dedicate sufficient time for the process.

#### Relevant concomitant care permitted or prohibited during the trial {11d}

In accordance with clinical standards, universally accepted intensive therapy is permitted and necessary during the trial. Standard medical procedures (not required by the protocol) can be performed by unit staff who are not part of the study team. No specific care is prohibited.

#### Provisions for post-trial care {30}

All participants will be monitored independently from the study according to clinical standards. Study follow-up will be continued for up to 90–104 days after randomization. There will be no additional provisions for post-trial care. No financial compensation will be provided to study subjects for their participation in the study. Participation in the study will not require the subjects to incur any additional expenses. The study subjects will be provided with all study procedures, including laboratory analyses and study visits, by the sponsor free of charge, and there will be no costs for the subjects while they are participating in the study. Should a study subject suffer a permanent injury directly related to the medication used or medical procedure performed in accordance with the study protocol, he/she is entitled to claim compensation from the mandatory civil liability insurance of the investigator and sponsor.

#### Outcomes {12}

Primary and secondary outcomes will be collected during the ICU stay and subsequent follow-up period at 3, 7, 28, and 90–104 days following randomization.

Primary endpoint

- Percentage of patients that achieved serum 25(OH) D3 levels ≥ 30 ng/ml on days 3 and 7 following vitamin D3 administration

Secondary endpoints

- Number of deaths on days 28 and 90–104 after vitamin D3 administration
- ICU treatment duration in patients administered with vitamin D3
- SOFA score on days 3 and 7 in patients administered with vitamin D3
- Catecholamine administration duration in patients administered with vitamin D3
- The length of CRRT use in hours from the time of study drug administration to the beginning of visit 4
- Total gastric residual volume in milliliters from the time of study drug administration to the date of the beginning of visit 3

Safety endpoints

- Percentage of patients with a toxic serum 25(OH)D3 levels (25(OH)D3 ≥ 150 ng/ml) on days 3 and 7
- Percentage of patients with a serum total calcium concentration > 11 mg/dl on days 3 and 7

#### Participant timeline {13}

The study flowchart id depicted in Fig. 1. Table 1 shows the participant timeline.

#### Sample size {14}

The sample size for study groups was calculated using statistical software R[1] version 4.1.1, with the following formula:

$$n_2 = \frac{\left(Z_\alpha + Z_\beta\right)^2}{(\varepsilon - \delta)^2}\left[\frac{p_1(1-p_1)}{k} + p_2(1-p_2)\right]$$

$$n_1 = k \cdot n_2$$

where:

$Z_\alpha$—critical value of the distribution for significance level.

$Z_\beta$—critical value of distribution for power (power = 1-$\beta$).

$\delta$—acceptable non-inferiority margin.

$\varepsilon$—difference in percentages of patients that reached an endpoint in groups.

$k$—sample size ratio between groups.

$p_1$—percentage of patients that reached target serum 25(OH)D3 levels ≥ 30 ng/ml on days 3 and 7 following vitamin D3 administration in the control arm.

$p_2$—percentage of patients achieving target serum 25(OH)D3 levels ≥ 30 ng/ml on days 3 and 7 following vitamin D3 administration in the treatment arm

Assuming that the percentage of patients who achieve the target levels of 25(OH)D3 is 50% in the control group (vitamin D3 at a dose of 500,000 IU) and 75% in the treatment group (vitamin D3 at a dose of 750,000 IU), then in order to achieve a power of 85% with a significance level $\alpha = 0.05$ and without an acceptable non-inferiority margin ($\delta = 0$), the study should enrol a minimum of 62 patients per group. Assuming a drop-out rate of approximately 10%, the number of recruited patients should be 138 (69 patients per group).

#### Recruitment {15}

Patients will be recruited by local investigators from the ICUs of the participating sites. Local investigators will be obliged to perform daily screenings in order to identify patients undergoing CRRT. Local investigators will be also obliged to report pre-screen failures on dedicated templates. They will report which CRRT patients were not included, and document the reasons why. A study subject for whom consent to participate in the study was obtained but who did not meet the inclusion criteria will be marked as a screen failure (SF) in the eCRF. Subjects who meet all the eligibility criteria but stop participating for other reasons (e.g., withdrawal of consent) will not be marked as a SF and the reason for their early withdrawal will be noted in the eCRF. The primary investigator will be alerted to each randomization and will keep track of the inclusion rate per site, in order to control the quality and efficacy of the recruitment process and to determine the optimal course of action with the local investigator as needed.

### Assignment of interventions: allocation

#### Sequence generation {16a}

A computer algorithm will be used to generate the random allocation sequence. Patients will be randomized in a 1:1 ratio. The study will use block randomization. The

[1] R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

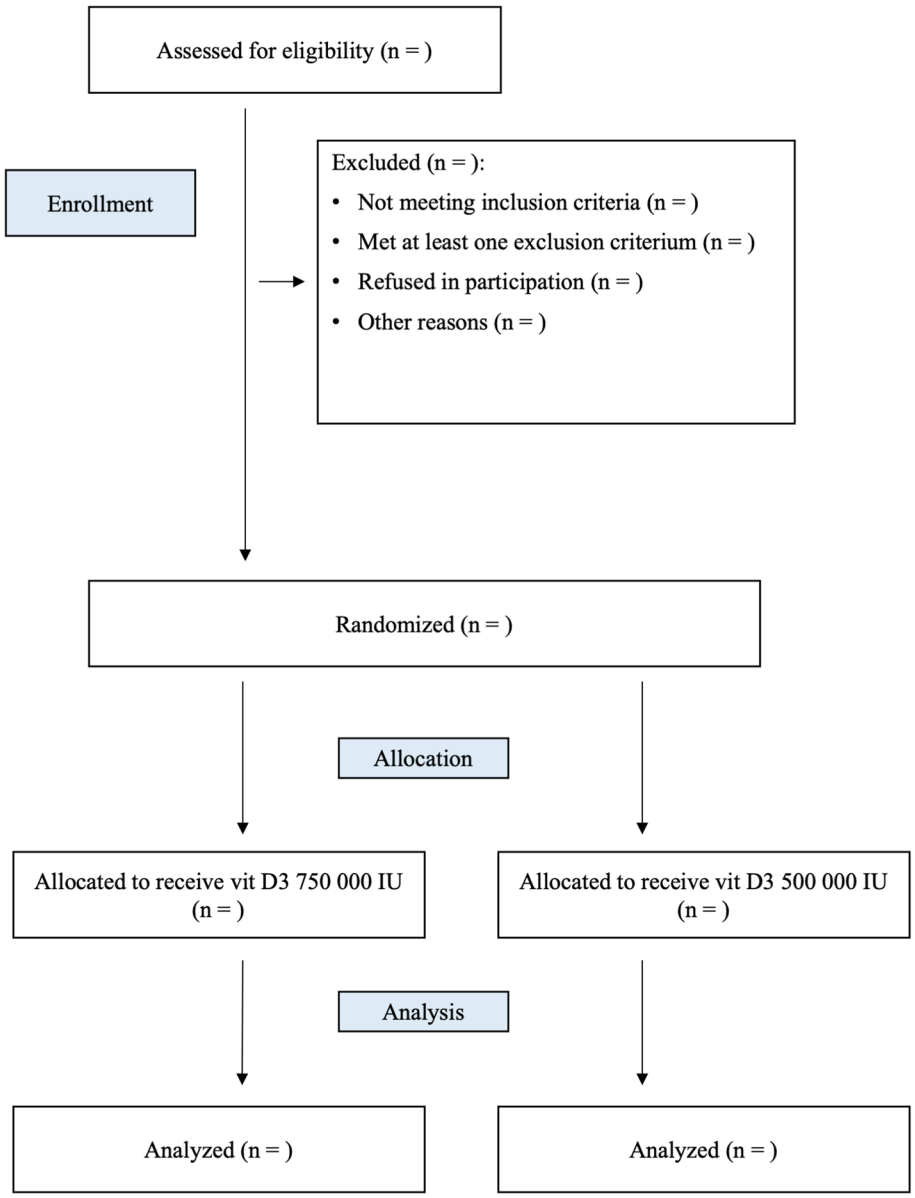


**Fig. 1** The study flowchart

selected randomization method is intended to ensure an equal number of patients in each study group.

#### Concealment mechanism {16b}

Randomization will be conducted centrally through an interactive web response system (IWRS). Allocation will be revealed to the investigator but not to the patient.

#### Implementation {16c}

After obtaining informed consent, the local investigators will use IWRS to allocate the patients to one of the study arms. There will be two arms: one interventional arm—single enteral administration of 750,000 IU of vitamin D3—and one control arm (standard therapy)—single enteral administration of 500,000 IU of vitamin D3.

**Table 1** The participant timeline

| Assessment criterion[a] | Visit 1 screening visit (screening, enrolment) | Visit 2 Day 0 Randomization, intervention (up to 24 h after V1) | Visit 3 Day 3 | Visit 4 Day 7 | Visit 5 Day 28 (± 4 days) | Visit 6 Day 90 up to day 104 |
|---|---|---|---|---|---|---|
| Informed consent process | x | | | | | |
| Comorbidities | x | | | | | |
| Concomitant treatment | x | x | x | x | x | x |
| 25(OH)D3 concentration | x[e] | | x | x | | |
| Inclusion/exclusion criteria | x | x | | | | |
| Randomization | | x | | | | |
| Adverse events | x | x | x | x | x | x |
| Basic information and demographic data | x | | | | | |
| SOFA score calculation | x | | x | x | | |
| SAPS II score calculation | x | | | | | |
| PTH testing | x[e] | | x | x | | |
| Complete blood count[b] | x[e] | | x | x | | |
| Clinical chemistry[c] | x[e] | | x | x | | |
| Serum pregnancy test (only women of child-bearing potential) | x[e] | | | | | |
| Assessment of results or performance of abdominal ultrasound/CT | x[f] | | | | | |
| CRRT parameters | x | | x | x | | |
| Vitamin D3 administration | | x | | | | |
| Survival status (mortality) | | | | | x | x |
| Assessment of ICU treatment duration | | | | | x | x |
| Collecting information on catecholamine use and ICU catecholamine administration duration | | | | | x | x |
| Collecting information on gastric residual volume (GRV) | | | x | | | |
| Collecting information on the length of CRRT | | | | x | | |
| Clotting parameters[d] | x[e] | | x | x | | |
| Physical examination | x | | x | x | | |

[a] *ICU*, intensive care unit; *PTH*, parathyroid hormone; *SAPS*, Simplified Acute Physiology Score; *SOFA*, Sequential Organ Failure Assessment

[b] Laboratory tests: white blood count (WBC), red blood count (RBC), hemoglobin, hematocrit, platelet count

[c] Laboratory tests: sodium, potassium, total calcium, chloride, phosphorus, urea, creatinine, bilirubin

[d] Laboratory tests: prothrombin time (INR), activated partial thromboplastin time (APTT)

[e] Possible use of data from tests performed prior to patient inclusion in the study, up to 72 h prior to visit 1

[f] Possible use of data from tests performed up to 37 days prior to the patient's admission to the ICU

## Assignment of interventions: blinding

### Who will be blinded {17a}?

Only the patients will be blinded. In view of the objective nature of the study endpoints, double blinding is not considered necessary in the NephroD study.

### Procedure for unblinding if needed {17b}

Due to the nature of the trial (single blinding), there is no need for an unblinding procedure.

## Data collection and management

### Plans for assessment and collection of outcomes {18a}

An individual patient's participation in the study will last approximately 90–104 days, and during this period, there will be 6 study visits.

### Visit 1 (screening)

Patients potentially meeting NephroD study inclusion criteria will be identified by investigators at the ICUs involved in the study. At the first study visit, patient

informed consent to participate in the study or consent of a guardianship court will be obtained. The investigator will review the patient's medical history and all available imaging and laboratory tests. Then, on the basis of an interview or the available medical records, basic information and demographic data will be collected, i.e., gender, age, body weight, height, body mass index (BMI), diagnosis on admission to the ICU, comorbidities, concomitant treatment. Vital signs (blood pressure, heart rate) will be assessed. A physical examination will be performed (abdominal cavity, respiratory system, nervous system, cardiovascular system, genitourinary system), and CRRT parameters will be collected, i.e., blood flow rate, pre-blood pump flow rate (PBP), dialysate flow rate, replacement fluid flow rate, net fluid removal, CRRT dose, CRRT method. The first visit also includes basic blood tests, i.e., complete blood count—white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count; clinical chemistry—sodium, potassium, total calcium, chloride, phosphorus, urea, creatinine, and bilirubin; and clotting parameters INR, APTT, serum 25(OH)D3, and PTH levels. In women of childbearing potential, a serum pregnancy test will be performed.

The results of abdominal ultrasound or abdominal computed tomography (CT) scans will be evaluated to rule out kidney stones. If an ultrasound or CT scan (both obtained within the last 37 days) is not available, a radiology specialist or internal medicine specialist with extensive experience in abdominal ultrasound diagnostics will perform an abdominal ultrasound scan. SOFA and SAPS II scores will be calculated and the data will be recorded in the patient's medical record. Imaging scans (ultrasound, abdominal CT) are permitted if performed within 37 days before the first visit and laboratory tests (complete blood count, clinical chemistry, clotting parameters, serum 25(OH) D3 and PTH levels) performed within 72 h before the first visit. The investigator will decide whether or not to repeat the imaging examinations and/or laboratory tests during the first visit based on the patient's current clinical status. During the screening visit, information on the occurrence of possible adverse events will also be collected.

#### Visit 2 (day 0)

Visit 2 needs to take place within 24 h following the completion of visit 1. Visit 2 involves reassessing the inclusion and exclusion criteria, a repeated verification of the informed consent, or consent of the competent guardianship court, randomization, and enteral administration of the assigned vitamin D3 dose.

#### Vitamin D3 administration

The drug will be administered by the ICU nurse at the study site who is a member of the study team. Before administering the drug, the investigator will check the accuracy of the prepared dose. The drug will be administered via a nasogastric tube or orally in situations where the patient does not have a gastric tube and is fed orally. The calculated dose of the vitamin D3 product will be given with water at a ratio of 1:1. During visit 2, information on possible adverse events and data on concomitant treatment will be collected.

#### Visit 3 (day 3)

Visit 3 includes measurement of blood pressure and heart rate, physical examination of the patient (abdominal cavity, respiratory system, nervous system, cardiovascular system, genitourinary system), and collection of CRRT parameters, i.e., blood flow rate, PBP, dialysate flow rate, replacement fluid flow rate, net fluid removal, CRRT dose, and CRRT method. The third visit also includes basic blood tests and clinical chemistry as well as clotting parameters, serum 25(OH)D3, and PTH levels. The SOFA score will also be calculated, and information will be collected on GRV from the time of the vitamin D3 administration to the beginning of visit 3. Information on possible adverse events and data on concomitant treatment will also be collected.

#### Visit 4 (day 7)

Visit 4 includes measurement of blood pressure and heart rate, physical examination of the patient (abdominal cavity, respiratory system, nervous system, cardiovascular system, genitourinary system), collection of CRRT parameters, and method. The fourth visit also includes basic blood tests, clinical chemistry, clotting parameters, serum 25(OH)D3, and PTH levels. The SOFA score will also be calculated, and information will be collected on the length of CRRT use from the time of the vitamin D3 administration to the beginning of visit 4. Information on possible adverse events and data on concomitant treatment will also be collected.

#### Visit 5 (day 28 (± 4 days))

Visit 5 will be held either at the hospital, involving a patient examination by a member of the study team, or as a telephone conversation with the patient or the patient's family member. The patient's survival status and the length of treatment at the ICU (in days) will be recorded together with information on the patient's intake of catecholamines and the length of catecholamine use (in days). Information on possible adverse

events and data on concomitant treatment will also be collected.

#### Visit 6 (day 90 to day 104)

Visit 6 will be held either at the hospital, and include an examination of the patient by a member of the study team, or as a telephone conversation with the patient or the patient's family member. The patient's survival status and ICU treatment duration in days will be recorded together with information on the patient's intake of catecholamines and the duration of catecholamine use in days. Visit 6 can take place between day 90 and day 104. Information on possible adverse events and data on concomitant treatment will also be collected.

### Plans to promote participant retention and complete follow-up {18b}

Conscious patients will receive extensive information regarding the study including risks and potential advantages. Local investigators will be obliged to make a maximum effort to carefully complete the study follow-up assessments. The importance completing of the follow-up will be emphasized. Telephone calls with patients or their proxy will be made to collect as much outcome data as possible. Reasons for drop-out or not attending follow-up visits will be reported. Patients are allowed to withdraw consent at any time during the study and are not obliged to give a reason for discontinuing. In case of study withdrawal, all data collected up until the moment of withdrawal will be retained unless objections are made by the participant or their proxy. In that case, all the data collected will be deleted.

### Data management {19}

The term eCRF used in this protocol should be understood to mean the electronic case report form on which the study team enters data previously collected on paper documents.

The eCRF is required by the sponsor and must be completed for every subject enrolled in the study (for whom consent to participate in this study was obtained). The eCRF is owned solely by the sponsor and must not be made available in any form to third parties—except for authorized representatives of the Sponsor or relevant regulatory authorities.

The investigator is responsible for ensuring that data entered in the eCRF are only based on patient source documentation. The investigator is also obliged to ensure that the data entered are accurate, complete, and up to date. The investigator or sub-investigator is responsible for resolving queries about missing/incomplete/erroneous data. Any corrections introduced to the entries in the source documentation must be dated, initialed, and explained (if necessary) and must not obscure the original entry.

In the final report, adverse events and medical/surgical history will be classified according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) in the eCRF system. Concomitant medications will be recorded in the database using codes from the WHO Model List of Essential Medicines based on the Anatomical Therapeutic Chemical Classification System.

Coding will be performed by a group of experts selected by the sponsor. Queries regarding inconsistent, implausible, or missing data will be reported in the eCRF. The data will be reviewed according to the GCP, verified, signed, and blocked, and the database will be locked. The investigator will confirm with his/her signature the completeness of each patient's data in the eCRF. After the study is completed, the eCRF will be archived, and a copy will be sent for archiving in the investigator file.

All original signed informed consent forms, adverse event/pregnancy report forms, source documents, and detailed treatment records as well as records of relevant correspondence (e.g., emails, minutes from meetings) should be archived by the investigator for a period set forth in the ICH guidelines, local regulations, or the study agreement, whichever is longest.

If for any reason, the investigator is unable to maintain the clinical study documentation for the required period of time (e.g., due to retirement or relocation), the sponsor should be notified in advance. Clinical study records must then be handed over to an appointed person accepted by the sponsor, e.g., another investigator, sub-investigator, another institution or an independent third party appointed by the sponsor.

The Investigator Site File must be stored for at least 5 years after the clinical study has ended or been discontinued, or longer, if required by the regulations.

The investigator must obtain the sponsor's written consent before deleting/destroying any files, even if the archiving requirement has already been fulfilled.

### Confidentiality {27}

All patient-identifying records will be considered confidential and will not be made public to the extent permitted by the applicable law. Study subjects will only be identified by unique ID numbers in study records and in the eCRF.

By signing the study protocol, the investigator undertakes to make every effort to keep patient data confidential and anonymous. All source documents of study subjects will be kept as confidential records in a secure place at the study site, in accordance with the Personal Data Protection Act.

The informed consent form will explain to the study subjects that sponsor representatives, independent ethics committees (IECs), or regulatory authorities are entitled to access medical records of the subjects for the purpose of verifying the information collected. All personal data made available to them will be treated with strict confidentiality in accordance with the applicable personal data protection laws.

Patient identities will not be disclosed in any publication resulting from this study. By signing this protocol, the investigator confirms that all information obtained by him/her in connection with the study will be considered confidential and will not be used for any purposes other than outlined herein. The above commitment will survive study termination.

#### Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

N/A. No collection, laboratory evaluation, or storage of biological specimens for genetic or molecular analysis is planned in this trial.

## Statistical methods

#### Statistical methods for primary and secondary outcomes {20a}

***Primary endpoint***

The analysis will test the null hypothesis that the percentage of patients that achieved the target serum concentration of 25(OH)D3 ≥ 30 ng/ml on days 3 and 7 post vitamin D3 administration is the same in both study groups. To this end, a proportion test will be used to compare the percentage of patients that achieved the target 25(OH)D3 levels in the two groups treated with two different vitamin D3 (25(OH)D3) supplementation doses: 500,000 IU and 750,000 IU, administered as a single enteral or oral dose in ICU patients undergoing CRRT and diagnosed with severe vitamin D3 deficiency. In addition, 95% confidence intervals will be defined. As part of the supplementary analysis for the primary endpoint, percentages and proportion differences will be calculated for serum 25(OH)D3 levels ≥ 30 ng/ml separately for day 3 and day 7 following vitamin D3 administration. Also, the primary endpoint will be analyzed together with imputed data for patients whose serum vitamin D3 levels at day 3 or 7 are unavailable. The imputation will be carried out as follows: if data are missing for both these timepoints or for day 3 only, the patient will be considered not to have achieved the primary endpoint. If a patient has data available for day 3 but not for day 7, the day 3 data will be used to impute day 7 data (i.e., the figures will be assumed to be the same at both timepoints), and the achievement of the primary endpoint will be assessed on this basis.

***Secondary endpoints***

The endpoints regarding the number of deaths on days 28 and 90 following vitamin D3 administration will be characterized with percentages and 95% confidence intervals. The duration of ICU treatment and catecholamine administration in patients supplemented with vitamin D3 will be summarized with the use of descriptive statistics. The median time to discharge from ICU and termination of catecholamine administration will be determined with the Kaplan–Meier method. SOFA scores at days 3 and 7 in patients supplemented with vitamin D3 will be summarized with the use of descriptive statistics. The relationship between the duration of CRRT use and serum vitamin D3 concentration will be analyzed using Poisson regression with the randomized study arm, 25(OH) D3 concentration measured at visit 4, and gender and age as explanatory variables. The results will be presented as model coefficient values with 95% confidence intervals. The relationship between GRV in milliliters from the time of drug administration to the date of the beginning of visit 3 and serum vitamin D3 levels in both study arms will be analyzed using a linear regression model. The selection of explanatory variables and the presentation of data will be identical to the model described above.

***Safety analysis***

Reported adverse events (and medical history) will be coded using MedDRA and summarized with descriptive statistics by system organ class (SOC) and preferred term (PT). Both the number of events and patients will be reported, including details of severity and causality. Endpoints related to the number of patients who develop toxic serum vitamin D3 levels (25(OH)D3 ≥ 150 ng/ml) on days 3 and 7 and the number of patients who achieve serum total calcium concentration > 11 mg/dl on days 3 and 7 will be characterized using percentages and 95% confidence intervals. Laboratory test results (hematology, biochemistry, coagulation parameters) will be reported as descriptive statistics and categorized (as above normal, normal and below normal) in shift tables versus the screening visit. Concomitant medication will be coded using the WHO Drug Dictionary and presented as counts and percentages of patients taking drugs classified as anatomical therapeutic chemical classification (ATC) ATC 2nd and ATC 4th levels.

#### Interim analyses {21b}

Upon recruitment of 50% of the target patient population, a treatment safety analysis will be conducted by an independent committee appointed by the Institute of

Medical Sciences of the University of Opole. The analysis will focus on potential toxicities associated with high-dose vitamin D3 supplementation. The committee responsible for the safety analysis will be empowered to terminate the study at this stage. The above analysis will not include efficacy measures.

#### Methods for additional analyses (e.g., subgroup analyses) {20b}

Additional analyses for primary and secondary endpoints will be conducted by subgroups defined by:

- Gender
- Age: $< 65, \geq 65$ years
- BMI: $< 30, \geq 30$
- CRRT modality: CVVHDF, CVVHF

#### Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

The primary outcome will be assessed using an intention-to-treat analysis. The primary endpoint will be analyzed together with imputed data for patients whose serum vitamin D3 levels at day 3 or 7 are unavailable. The imputation will be carried out as follows: if data are missing for both these timepoints or for day 3 only, the patient will be considered not to have achieved the primary endpoint. If a patient has data available for day 3 but not for day 7, the day 3 data will be used to impute day 7 data and the achievement of the primary endpoint will be assessed on this basis.

#### Plans to give access to the full protocol, participant-level data and statistical code {31c}

Granting public access to the full study protocol and datasets used and/or analyzed is not planned. However, the primary investigator will be able to grant such access upon reasonable request and consent given by the sponsor.

## Oversight and monitoring

#### Composition of the coordinating center and trial steering committee {5d}

The Opole University Hospital is the sponsor of the trial and will act as overall and national coordinating center and will coordinate the day-to-day management of the trial (Dr. Tomasz Czarnik).

#### Trial steering committee

Dr. Tomasz Czarnik (primary/chief investigator/coordinator).

Dr. Anna Wludarczyk, Dr. Ryszard Gawda, Dr. Pawel Piwowarczyk, Dr. Michal Borys, Dr. Szymon Bialka (local investigators).

Prof. Wojciech Szczeklik, Prof. Miroslaw Czuczwar, Prof. Hanna Misiolek (experts).

The trial steering committee oversees the conduct of the trial and advises the trial sponsor on matters of trial execution and management.

#### Data management team

Biostat Sp. z o. o. is the clinical research organization (CRO) responsible for trial data management including construction and maintenance of the eCRF, data validation, and data monitoring.

Milosz Bielski is the independent study monitor.

The independent study monitor will perform on-site visits at regular intervals to assess the overall quality of the study and compliance with the protocol, ranging from the informed consent procedure to source document verification.

#### Composition of the data monitoring committee, its role and reporting structure {21a}

Upon recruitment of 50% of the target patient population, a treatment safety analysis will be conducted by an independent data monitoring committee appointed by the Institute of Medical Sciences of the University of Opole. The analysis will focus on potential toxicities associated with high-dose vitamin D3 supplementation. The committee responsible for the safety analysis will be empowered to terminate the study at this stage. The above analysis will not include efficacy measures.

#### Adverse event reporting and harms {22}

Information on all adverse events (AEs), including serious adverse events (SAEs), and safety endpoints (where applicable) will be collected and recorded in the source documentation of the subjects in the study and in the eCRF, regardless of their causal relationship. All safety data emerging in relation to study procedures will be reported to the sponsor/investigator: AEs and SAEs via the eCRF. Once the SAE form has been saved by the investigator, it will be automatically sent from the eCRF to the person responsible for safety and to the sponsor. Every SAE must be reported by the investigator within 24 h of collecting minimum information. If an SAE occurs, a member of the study team at the affected site must fill in the relevant form in the eCRF system. All SAE information will be collected and recorded in accordance with the information contained in the "Serious Adverse Event Reporting Form." The investigator must assess the relationship of the event with the medicinal product, its severity, and serious/non-serious

status, as well as provide a description of the event, attaching relevant documents.

#### Frequency and plans for auditing trial conduct {23}

During the study, the sponsor or a designee will perform regular monitoring visits to ensure compliance with the protocol and ICH GCP guidelines. The representative of the sponsor will have access to source documentation in order to verify the accuracy of the data entered in the eCRF.

#### Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Any protocol amendments introduced after the approval from the independent ethics committee (IEC) will be submitted to the committee in accordance with applicable regulations. The investigator will immediately notify the IEC of any unexpected issues or protocol deviations that could present a risk to study subjects or other individuals involved in the study. The investigator will not make any modifications to the study or to its progress without prior consent from the IEC, unless it is necessary in order to eliminate any direct danger to the patients. The principal investigator will provide the IEC with a study report after the study has been completed.

#### Dissemination plans {31a}

The clinical study report will be developed according to ICH Guideline E2 (Structure and Content of Clinical Study Reports) and the applicable local and international law. The study data will be the property of the sponsor. The sponsor reserves the exclusive right to publish the data.

Before any documents are submitted for publication or presentation, the investigator must obtain approval from the sponsor for all the documents to be submitted. This will allow the sponsor to ensure the protection of proprietary information and to make comments based on information from other studies that might not yet be available to the investigator.

Any inventions and resulting patents, improvements, and/or know-how generated on the basis of the study data will be and remain the sole property of the sponsor, unless agreed otherwise.

A description of this clinical study, including a summary of the results, will be available on relevant websites, including www.ClinicalTrials.gov and www.clinicaltrialsregister.eu.

## Discussion

The most challenging part in the execution of the trial will be to obtain the informed consent in unconscious patients. According to Polish law, consent will be obtained on the basis of consent from a guardianship court. The entire procedure needs meticulous cooperation between local investigators and local courts. Regarding follow-up, local investigators will be urged to perform comprehensive follow-up visits and collect as much data as possible. This entails telephone calls and on-site visits for still hospitalized patients. The principal investigator and CRO will regularly monitor recruitment progress, the protocol adherence, and AE/SAE reporting and make any changes as needed.

## Trial status

Ethical approval was signed on 19 January 2022 by Komisja Bioetyczna Opolskiej Izby Lekarskiej w Opolu, 45–054 Opole, ul. Grunwaldzka 23; L. dz. OIL/KB/344/22. The study protocol version 4.0 was approved by ethics committee on 19 October 2023 (Komisja Bioetyczna Opolskiej Izby Lekarskiej w Opolu, 45–054 Opole, ul. Grunwaldzka 23; L. dz. OIL/KB/200/23). The first participant was included on 20 December 2022. The recruitment process is estimated to be completed around August 2025.

#### Abbreviations

| | |
|---|---|
| AE | Adverse event |
| ALT | Alanine aminotransferase |
| APTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATC | Anatomical therapeutic chemical |
| BMI | Body mass index |
| CRO | Clinical research organization |
| CRRT | Continuous renal replacement therapy |
| CT | Computed tomography |
| CVVHDF | Continuous veno-venous hemodiafiltration |
| CVVHF | Continuous veno-venous hemofiltration |
| DBP | Vitamin D binding protein |
| ECCO2R | Extracorporeal carbon dioxide removal |
| ECMO | Extracorporeal membrane oxygenation |
| eCRF | Electronic case report form |
| ESPEN | European Society of Clinical Nutrition and Metabolism |
| GCP | Good clinical practice |
| GRV | Gastric residual volume |
| ICH | International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| ICU | Intensive care unit |
| IEC | Independent ethics committee |
| INR | International normalized ration |
| IWRS | Interactive web response system |
| MedDRA | Medical Dictionary for Regulatory Activities |
| PBP | Pre-blood pump flow rate |
| PT | Preferred term |
| PTH | Parathormone |
| RBC | Red blood count |
| SAE | Serious adverse event |
| SAPS II | Simplified Acute Physiology Score |
| SF | Screen failure |
| SOC | System organ class |

| | |
|---|---|
| SOFA | Sequential Organ Failure Assessment |
| WBC | White blood count |
| WHO | World Health Organization |
| VDR | Vitamin D receptor |

**Acknowledgements**
N/A

**Authors' contributions {31b}**
TC is the principal investigator; he conceived the study, obtained funding, and developed the protocol. TC and RG drafted the manuscript. WS, MC, and HM provided methodological and content-related expertise during protocol development. RG, AW, SB, MB, and PP are local investigators of participating study sites. All co-authors critically reviewed the trial protocol. All authors read and approved the final version of the manuscript.

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**Data availability {29}**
The datasets used and/or analyzed during the trial will be made available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate {24}**
Ethical approval was signed on 19 January 2022 by Komisja Bioetyczna Opolskiej Izby Lekarskiej w Opolu, 45–054 Opole, ul. Grunwaldzka 23; L. dz. OIL/KB/344/22. All study participants will provide written informed consent in accordance with the description provided above.

**Consent for publication {32}**
N/A.

**Competing interests {28}**
The authors declare that they have no competing interests.

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## Publisher's Note