Efficacy of high dose versus low dose vitamin D supplementation on serum levels of inflammatory factors and mortality rate in severe traumatic brain injury patients: study protocol for a randomized placebo-controlled trial

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Study protocol

Keywords: traumatic brain injury, vitamin D, inflammation, mortality

Posted Date: April 3rd, 2020

DOI: https://doi.org/10.21203/rs.2.16302/v3

Version of Record: A version of this preprint was published on July 29th, 2020. See the published version at https://doi.org/10.1186/s13063-020-04622-6.

Abstract

Background Traumatic Brain Injury (TBI) is the most common trauma worldwide and is a leading cause of injury-related death and disability. Inflammation is a major problem among TBL patients which is in association with severity of illness and mortality in brain trauma patients, especially in subdural hemorrhage and epidural hemorrhage cases. A high percentage of adults admitted to the intensive care unit with critical conditions are diagnosed with vitamin D-deficiency, this deficiency may induce impaired immune responses and increase the risk of infections. Vitamin D intervention has been shown to modulate pro- and anti-inflammatory cytokines in non-critically ill patients, but to date, there is no substantial data on the effectiveness of vitamin D for the improvement of immune function in traumatic brain injury patients.

Methods/design A randomized clinical trial (RCT) will be performed on 74 Iranian adults 18-65 years old with brain trauma, and will be treated daily by vitamin D supplements (100000 IU oral drop) or a similar placebo (1000 IU) for 5 days.

Discussion If this randomized clinical trial elucidates reduction in inflammatory cytokines, it would provide the evidence for multi-central clinical trials to evaluate the efficacy of vitamin D supplementation in neuro -critically ill patients. Since vitamin D supplements are inexpensive and safe, this clinical trial could have the potential to improve clinical outcomes in traumatic brain injury patients through reduction of inflammation and infection associated morbidity and mortality rates.

Background

Vitamin D is an essential hormone for calcium homeostasis and its intestinal absorption, it also has an important role in several neuromuscular activities and metabolic responses (1). Recent studies indicated that the active form of vitamin D may have a crucial function in modulating immune responses to inflammatory conditions and infectious diseases (2, 3)**.** According to previous data, in critically ill patients' vitamin D deficiency has been associated with a high incidence of adverse events and mortality (4). Vitamin D can induce production of anti-inflammatory cytokines and some antimicrobial proteins such as cathelicidin by macrophages and neutrophils (3). Antimicrobial proteins are expressed significantly in the respiratory epithelium and other organs and they could reduce risk of infections in integumentary barrier sites (5, 6)**.** Notably, infectious diseases such as pneumonia and sepsis are common in traumatic patients leading to more than 20 % rate of death in these patients, in addition there is a high prevalence of vitamin D deficiency in these patients, therefore vitamin D treatment is of high importance in traumatic brain injury (TBI) patients (7-9). Moreover, vitamin D deficiency is also related to serious complications such as coma, slow neurological recovery and critical illness polyneuropathy in TBI patients (TBI) (10, 11). Although, there has been a direct association between vitamin D deficiency and adverse clinical outcomes, the data regarding vitamin D supplementation from randomized clinical trials are limited (12). It has not yet been shown that vitamin D supplementation can efficiently affect the endpoints of TBI patients (4, 9). The type of vitamin D supplement, rout of intervention and speed of

normalization may be associated in clinical outcomes (4, 9, 12). As compared to vitamin D injection, enteral vitamin D administration seems to be more effective and is considered safe (13, 14). To date, studies on the effects of vitamin D on brain trauma patients have been inadequate and could not provide definitive conclusions (15-17). The purpose of the present study is to determine the effect of high dose vitamin D (Cholecalciferol) supplementation versus low dose on mortality rate and inflammatory cytokines levels, in critically ill traumatic brain patients under enteral nutrition therapy at intensive care unit (ICU).

Methods/design

Study design

The treatment of vitamin D deficiency in neurocritical ill patients (VITdAL-ICU) protocol is designed according to the CONSORT guidelines for randomized, clinical trial (RCT) (18). The protocol of this RCT is approved by Medical Ethics Committee of Mashhad University of Medical Science (IR.MUMS.MEDICAL.REC.1397.381) and is registered at the Iranian Registry of Clinical Trials under IRCT20180619040151N3. The flow diagram illustrates details of the current study protocol in Figure 1. Moreover, the study time framework for screening, supplementation and monitoring are described in Figure 2.

Study objectives and rational

The primary goal of the present study is to investigate the effect of daily intake of high dose vitamin D (Cholecalciferol) supplements (100000 IU) versus low dose vitamin D supplements (1000 IU) on mortality rate and inflammatory markers through a double-blind, randomized, controlled clinical trial on traumatic brain injury (TBI) patients. According to previous observational studies, TBI patients with vitamin D deficiency had higher mortality rate and neuro-inflammation in comparison with TBI patients with vitamin D sufficiency (15, 17). Also, as vitamin D deficiency is highly prevalent in critically ill patients, rapid normalization of this deficiency may be beneficial to these patients (7, 22, 23). For this reason, we hypothesized that rapid correction of vitamin D deficiency in TBI patients may improve inflammation and decrease mortality rate. This study will also evaluate seven secondary outcomes that may be influenced by vitamin D administration in neurocritical patients. In order to determine the effects of high dose vitamin D intervention 1) Glasgow coma scale (GCS), 2) time of final weaning 3) time of discharge from ICU 4) SOFA score (Sequential Organ Failure Assessment), 5) APACHE II Score (Acute Physiology and Chronic Health Evaluation II) 6) delirium severity and 7) parathyroid hormone (PTH) will be assessed in the treatment group compared to control group. The study participants will be recruited from adult wards at trauma referral hospitals namely Kamyab and Taleghani in Mashhad, Iran. This study is a phase II-RCT and it has started from August 2019 and will carry on for 5 months.

Eligibility criteria

Eligibility criteria for TBI patients are listed below. Participants will be allocated into four equal blocks by random in two groups using an online randomization list.

Inclusion criteria

1-Patients: traumatic brain injury adults aged 18-65 years' old.

2-Admission to neurocritical care unit with Epidural hemorrhage (EDH) and subdural hemorrhage (SDH). Because, these two types of trauma are most prevalent in patients admitted to our intensive care unit.

3-Glasgow Coma scale 7-9

4-TBI patients with vitamin D (25-hydroxy vitamin D3) levels lower than 20 ng/ml

5-Recived informed consent from patient's parents or family members prior to intervention

Non-entry criteria

1- Patients with hypercalcemia (Ca > 10.8 mg/dl)

2-TBI Patients who are NPO more than 48 hours or have started total parenteral nutrition (TPN).

3-Severe and active bleeding

4-Patients treated with inotropic and corticosteroid drugs.

5- Patients treated with therapeutic dose of any vitamins and minerals apart from the routine ICU protocol

6-Patients with body mass index (BMI) > 40kg/m^2 and BMI < 17kg/m^2

7-History of any disease such as autoimmune disorders, cancer, sepsis, infection, liver disorder, kidney disorder, diabetes, heart failure and metabolic diseases

8-Known pregnancy and lactation

9-Patients who are transferred from other ICUs after > 1week

Exclusion criteria

Death before 7 days of traumatic brain injury patients

Request to stop the study by patients' parents or family members

Patients treated with different medication protocol

If item 1 to 5 of non-entry criteria are met

Study design and setting

We will include 74 TBI patients with Epidural hemorrhage and subdural hemorrhage as diagnosed according to computed tomography scan (CT) or Magnetic resonance imaging (MRI) findings by the neurosurgeon. Patients will receive vitamin D (Cholecalciferol) drop (100000 IU) or 1000 IU identical control drop in a 1:1 ratio through random assignment method. Vitamin D drops are manufactured by Zahravi Company under good manufacturing practice (GMP). Each vial of vitamin D drop is produced to be dissolved completely in 1ml of extra virgin olive oil (Familia Company), through a nasogastric tube (NGT) for 5 days in TBI patients. Both high dose and low dose drops will have the same bottle, flavor and aroma to the blinded.

Sample size

To calculate the sample size, in accordance with a previous study (7), interleukin 6 (IL-6) will be used as the main variable and the type one error is considered with an alpha of 0.05. We estimated 62 TBI patients with 80% power to detect an effect size of 0.5 for reduction of IL-6 between intervention and control groups as calculated with the formula shown below. Considering 20% dropouts in this study, the total sample size is estimated to be 37 participants in each group as calculated in below formula (7).

[Please see the supplementary files section to view the equation.]

Randomization and blinding

Block randomization method will be considered for this trial study. TBI patients will be randomized (in four blocks) into intervention group and control group based on a blinded randomization list generated by randomization and online databases for clinical trials (https://www.sealedenvelope.com) and will be managed by research director of Clinical Nutrition Department, Mashhad University of Medical Sciences. Patients will be allocated by random according to the severity of brain injury (GCS 7-8 and 8-9), type of brain injury (EDH and SDH) and gender to ensure match distribution of these factors in all four blocks. Given that ICU supervisor has no knowledge of which vial contains high dose or low dose of vitamin D, TBI patients will be randomized to group A or B. The label A or B on vials will be deleted by the research director before allocating the study supplements, thus the medication will appear similar to study researcher and nurses. The TBI patient's study identification code will be documented and intervention group may only be determined by comparing the patient's study number to the reference blinded list, which only research director will have access to until the trial is finished. Figure 2 indicates the SPIRIT schedule of evaluations and interventions.

Proposed analysis

To determine the normal distribution of variables, Kolmogorov-Smirnov test will be conducted. The analysis will be performed according to intention-to-treat (ITT) test. One-way analysis of variance (ANOVA) or multi-variable covariance analysis (ANCOVA) will be conducted to examine differences in severity of brain injury score (GCS), APACHEII score, SOFA score, delirium score and all variables at study

baseline between the two groups. If the distribution of variables turns abnormal in this study, Kruskal-Wallis test will be performed to compare the case and control groups, and Wilcoxon test will be carried out for inter-groups comparison. The P values less than 0.05 will be considered statistically significant.

Primary efficacy endpoint

The primary efficacy outcome for this trial will be assessed by comparing the changes in vitamin D levels and inflammatory markers (IL-6, Monocyte Chemoattractant Protein-1 (MCP-1) and C - reactive protein (CRP)) between two groups. The rate of mortality in TBI patients until 28 days after admission (in ICU and regular ward) will be assessed in high and low dose vitamin D supplement groups. The impact of high dose versus low dose of vitamin D will be analyzed, with and without adjustment for age, sex, type of brain injury and severity of injury. If the results differ after adjusting for the variables, the results of both analyzes will be reported.

Secondary efficacy endpoints

All analyses will be conducted with and without adjustment for age, sex, type of brain injury and severity of the injury. Time to event analysis will be analyzed similarly as the primary endpoint. The secondary outcomes are listed below:

a. Comparison of GCS score changes between intervention and control groups during intensive care unit stay
b. Comparison of APACHE II and SOFA score between intervention and control groups during intensive care unit stay.
c. Comparison of incidence of delirium between intervention and control groups during intensive care unit stay.
d. Comparison of changes in PTH levels between intervention and control groups during intensive care unit stay.
e. Comparison of time of discharge from intensive care unit in patients treated with high dose and low dose vitamin D groups (during the first 28 days of admission).
f. Comparison of time to weaning from mechanical respiratory support in patients treated with high dose and low dose vitamin D groups (during the first 28 days of admission).
g. Comparison of need for a tracheostomy between intervention and control groups during intensive care unit stay.
h. Comparison of occurrence of infections among intervention and control groups during intensive care unit stay.

Study assessments

At the beginning of the trial, demographic information, anthropometric parameters (weight (bed scale, Balas company), (estimating height from ulna length), mid arm circumference (measured at the mid-point

between the tip of the shoulder and the tip of the elbow), fat free mass, fat mass and (bioelectrical impedance analysis s10, InBody company), vitamin D3 status (25-hydroxy vitamin D3, ELISA kit) , IL-6 (ELISA kit) , MCP-1(ELISA kit), quantitative CRP (ELISA kit), PTH (ELISA kit) , Ca, APACHE II score, SOFA score and delirium score will be measured and repeated on day 7 and 14 after intervention in groups A and B. All drugs will be ordered daily upon physician's request during ICU stay and will be recorded. For adjusting the effect of energy on final outcomes, based on ESPEN guideline for all patients 25kcal/ kg actual body weight energy is considered (19). Moreover, the total kilocalories and macronutrient delivered by enteral feeding will be calculated daily for all the participants. Blood samples (5mL) will be collected at baseline (ICU admission) and 7 and 14 days after intervention at 10:00 am. Blood sample will be taken from veins and centrifuged at room temperature, then stored at -20 °C. Finally, the samples will immediately be transferred with the cold box to a freezer -80 °C for future inflammatory cytokines measurements. All routine biochemistry, hematology and urine test, until day 14, will be measured after sampling in Kamyab Hospital lab, Mashhad, Iran. Serum IL-6, MCP-1 and CRP levels will be analyzed in the baseline and day 7 and 14 at Immunology Lab of Bu Ali Research Institute, Mashhad, Iran. In addition, in case of occurrence of infections (blood stream, the urinary tract, the gastrointestinal tract, wounds and lung) an internist specialist will take responsibility (20). A summary of the schedule of registration, supplementation, and study evaluation is shown in Figure 3.

Safety

Vitamin D deficiency treatment is required for patients with low vitamin D levels (< 20 ng/ml) [54]. However, the primary side effect of Cholecalciferol supplementation is hypercalcemia (13). To prevent hypercalcemia in TBI patients, serum vitamin D and calcium levels are evaluated regularly, as adequate vitamin D levels are reached hypercalcemia is likely to be treated, therefore the intervention will be stopped (21). Moreover, to keep track of the patient's condition, daily vital signs and clinical examinations by neurosurgeon and ICU supervisor will be carried out and will be stopped if the patient's condition deteriorates.

Discussion

In the present study, if vitamin D significantly reduces the rate of mortality and inflammation in TBI patients, it can potentially provide the background for larger and well-designed multicenter clinical trial studies to establish the effect of vitamin D supplementation on TBI patients. Furthermore, vitamin D treatment is inexpensive and safe with minimal side effects, also could have positive impacts on critically ill patients (such as decrease mortality rate and hospital length) according to previous studies (7, 13, 14, 21, 24). Potential limitations of the proposed study are as follows: measurement of vitamin D3 concentration with the ELISA test instead of the gold standard method because of current economic problem. Blood transfusion (because of trauma and bleeding, some TBI patients may require blood transfusion during the study, which may affect the biochemistry outcomes. Albumin injection in hypo-albuminemia patients (it can affect the levels of serum albumin). Also, TBI patients may need to undergo other surgeries than brain surgery thus, this factor could affect the study outcomes. For this reason, we

will compare mortality rate and inflammatory cytokines levels in all participants (intervention and control) and separate patients with (anemia and hypoalbuminemia) in each group. If the rate of death or inflammation is different from those in the case or control group, the results will be reported separately. Based on recent studies, we have considered the suggested vitamin D dose (100000 IU/day for 5 days) by Han, *et al* (21)*.,* as it is shown to be safe and efficacious in critically ill patients. Therefore, we believe that present study could elaborate the necessity of vitamin D treatment and its efficacy on TBI patients at ICU.

Conclusion

The present clinical trial has begun on June 2019 and will be completed in November 2019. If comprehensive results are observed in the clinical outcomes of TBI patients, vitamin D treatment could be conducted as a new approach for optimizing the traumatic brain injury patients' care at ICU.

Trial Status

This trial (protocol version number version 1.1, November 13, 2017) is ongoing. Participant recruitment began on August 11, 2019, and completed on December 21, 2019. The trial procedures are expected to be completed by the end of January 2020.

Abbreviations

VITdAL-ICU: The correction vitamin D deficiency in critically ill patients

ICU: Intensive care unit

TBI: Traumatic brain injury

EDH: Epidural hemorrhage

SDH: subdural hemorrhage

EN: Enteral

TPN: Total parenteral nutrition

BMI: Body mass index

NPO: Nothing by mouth

RCT: Randomized controlled trial

GCS: Glasgow Coma Scale

APACHE II: Acute physiology and chronic health evaluation

SOFA: Sequential organ failure assessment

IL-6: Interleukin 6

MCP-1: Monocyte chemoattractant protein-1

CRP: C-reactive protein

PTH: Parathyroid hormone

Ca: Calcium

LOS: length of stay

Declarations

Acknowledgements

We would like to thank the vice chancellor of research center of Mashhad University of Medical Sciences for the sponsorship of this study, with thesis number: 961867 and ethical approval code: IR.MUMS.MEDICAL.REC.1397.381.

Authors' contributions

ASM, NA, SA and EM all contributed to the design of the present study protocol. SM, RH and RR collaborated to perform of the clinical trial and TH and RG promoted the statistical plan, which was approved by all the authors, and drafted the manuscript. All authors read and approved the final manuscript.

Funding source

This trial is funded by Mashhad University of Medical Sciences (grant # 961867).

Availability of data and materials

Final data for the present clinical trial can be made accessible upon email request. Interested researchers should contact Dr. Abdolreza Norouzy at Norouzya97@gmail.com.

Ethics approval and dissemination

Mashhad University of Medical Sciences, School of Medicine Research Ethics Committee (IR.MUMS.MEDICAL.REC.1397.381), has approved the study protocol. Written informed consent will be obtained from the parents or family members of the TBI patients at ICU. Study participants will be monitored at Shahid Kamyab and Shahid Taleghani referral hospitals based on ICU protocols, including

routine medical and nutrition therapy for severe neurocritical patients. If we intend to make any modifications on the protocol (e.g., changes to eligibility criteria, outcomes, analyses), we will inform the relevant parties (e.g., investigators trial registries, journals). The results of this trial will be published for the benefit of healthcare professionals and the public.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

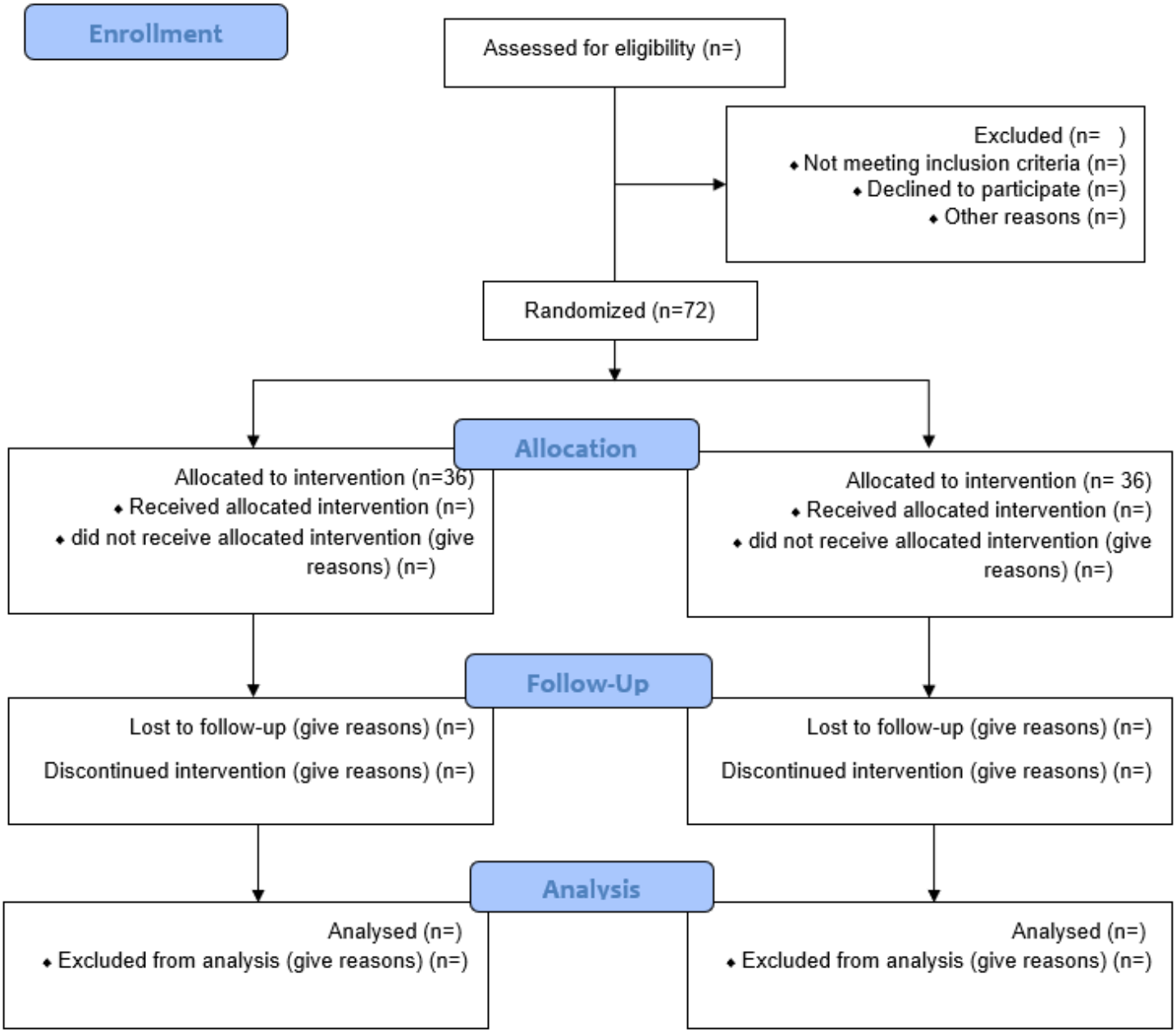

Figure 1

Participant flow diagram according to Consolidated Standards of Reporting Trials (CONSORT) 2010 statement

	Enrolment	Allocation		Post-allocation				
TIMEPOINT**	Day 1	Day 1	*Day 1*	*Day 5*	*Day 7*	*Day 14*	*Day 28*	*etc.*
ENROLMENT:								
Eligibility screen	X							
Demographic data and vitamin D levels	X							
Blood sample	X				X	X		
Allocation		X						
INTERVENTIONS:								
[high dose vitamin D]			←	—				
[low dose vitamin D]			←	—				
ASSESSMENTS:								
Primary : *[Vitamin D level, IL-6, MCP-1, CRP, PTH]			X		X	X		
Secondary: * [GCS, BMI, FFM, FM, APACH II, SOFA, delirium severity, infection rate, time to weaning from MV and discharge from ICU]			←	—	—	→		
[28 day mortality]			←	—	—	—	→	
Adverse events (Ca)			X		X	X		

Figure 2

SPIRIT figure showing schedule of interventions and assessments. IL-6: interlukine-6, MCP-1: monocyte chemoattractant protein-1, CRP: C-reactive protein, PTH: parathyroid hormone, GCS: Glasgow coma scale, BMI: body mass index, FFM: fat free mass, FM: fat mass, APACHEE II: acute physiology and chronic health evaluation, SOFA: Sequential Organ Failure Assessment, MV: mechanical ventilator

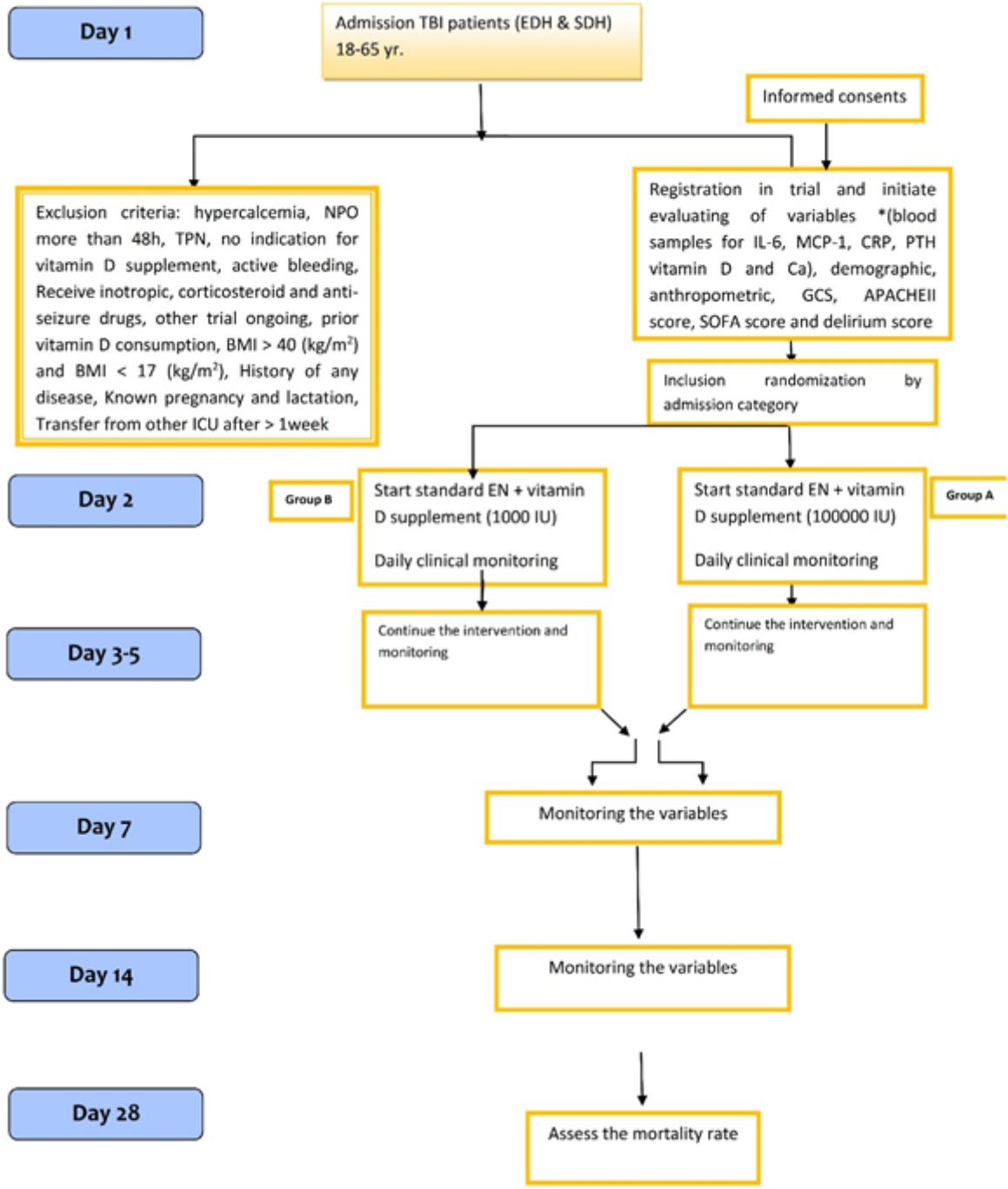

Figure 3

Trial procedures flow sheet

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Equation.docx
- SPIRITChecklistdownload8Jan13.pdf