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Protein Supplementation versus Standard Feeds in Critically Ill Children: A Dual-Centre Randomized Controlled Pilot Trial

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Abstract

Introduction

Protein-energy malnutrition, increased catabolism in critical illness and inadequate nutritional support leads to loss of lean body mass with muscle wasting and delayed recovery. However, there remains clinical equipoise regarding the risks and benefits of protein supplementation. This pilot trial will determine the feasibility of performing a larger multicentre trial to determine if a strategy of protein supplementation in critically ill children with body mass index (BMI) z-score ≤ -2 is superior to standard enteral nutrition in reducing the length of stay in the paediatric intensive care unit (PICU).

Methods and analysis

This is a randomized controlled trial of 70 children in two PICUs in Singapore. Children with BMI z-score ≤ -2 on PICU admission, who are expected to require invasive mechanical ventilation for more than 48 hours, will be randomized (1:1 allocation) to protein supplementation of ≥1.5g/kg/day in addition to standard nutrition, or standard nutrition alone for 7 days after enrolment or until PICU discharge, whichever is earlier. Feasibility outcomes for the trial include effective screening, satisfactory enrolment rate, timely protocol implementation (within first 72 hours) and protocol adherence. Secondary outcomes include mortality, PICU length of stay, muscle mass, anthropometric measurements and functional outcomes.

Ethics and dissemination

The trial protocol was approved by the institutional review board of both participating centres (Singhealth Centralised Institutional Review Board and National Healthcare Group Domain Specific Review Board) under the reference number 2020/2742. Findings of the trial will be disseminated through peer-reviewed journals and scientific conferences.

Trial registration number: NCT04565613

Strengths and limitations of this study

- To our knowledge, this is the first randomised controlled trial applying enteral protein supplementation to critically ill children
- There is no consensus on the optimal dose for protein intake during pediatric critical illness. Following recommendations of the American Society of Parenteral and Enteral Nutrition (ASPEN), this study will administer 1.5g/kg/day of protein to critically ill children. We also chose to focus our study on nutritionally high-risk patients (BMI z-score ≤ -2) who have the greatest potential to benefit from nutritional therapy.
- As the distribution of malnourished children (as defined by a BMI z-score ≤ -2) and PICU support/therapies are variable geographically, the study will employ randomization by centre to achieve balance in treatment allocation within each centre and account for centre-specific effects in the analysis.

Introduction

Background and rationale

Protein malnutrition is pervasive in paediatric intensive care unit (PICU) patients. Up to 40% of critically ill children have increased protein turnover and catabolism leading to protein malnutrition.(1-3) Increased catabolism of protein is likely attributable to a combination of various factors including critical illness inflammation, immobility and inadequate nutrition support.(4) Inadequate nutritional provision has been reported in several PICU studies, with reported rates of protein inadequacy ranging from 37 to 87%. (5-7) Inadequate protein intake is associated with poor clinical outcomes in critically ill children. In a large, multicentre cohort study, protein intake at or below 60% of the prescribed amount was associated with greater odds of mortality compared to those that received >60% of prescribed protein. (6) This was also demonstrated in critically ill children with acute respiratory distress syndrome (ARDS) requiring mechanical ventilation (MV) where children with protein intake of at least 1.5g/kg/day by day 3 of PICU stay had lower risk of mortality.(5) Other concerns of protein malnutrition include the loss of lean body mass with muscle wasting and subsequent functional disability, delayed MV weaning, prolonged hospital stay and increased mortality. (8, 9)

There is marked heterogeneity of patients admitted to the PICU. One subset of patients shown to be at high risk of increased morbidity and mortality are those who are underweight on PICU admission. (10) It is hypothesized that children who are underweight have reduced body stores and are thus at greater risk of nutritional decline in the event of nutrient inadequacy (10). As such, a targeted approach of protein supplementation in this particular group of patients can potentially lead to improved clinical outcomes. Thus far, there are no trials evaluating the benefits/risks of supplemental enteral protein administration to critically ill children, highlighting the presence of clinical equipoise.

Due to the inherent challenges of completing randomized controlled trials (RCTs) in pediatric critical care, a rigorous pilot RCT is crucial to evaluate the feasibility of a large RCT. A pilot trial may prevent pursuit of a trial that is ultimately not feasible. This pilot trial is a step towards the large trial needed to provide high-quality, compelling evidence required to develop guidelines for nutrition care in the PICU.

Objectives

The objectives of this pilot trial are to determine the feasibility, efficacy and safety of conducting a large multicentre RCT on protein supplementation in critically ill children. Feasibility related objectives include determination of the proportion of eligible patients approached for consent, likelihood of participants receiving their first protein supplementation within 72 hours of enrolment, participant accrual and protocol adherence. Since this is a pilot trial, efficacy objectives are secondary and will include a reduction in PICU mortality, length of stay, and an improvement in muscle mass, anthropometric measures and functional status at pre-determined follow-up intervals. Safety objectives include surveillance for adverse effects of protein supplementation—including feed intolerance, acute kidney injury, enterocolitis and other gastrointestinal related complications. This pilot trial will also refine inclusion and exclusion criteria, test study procedures, streamline data collection, and assess parental and physician acceptance of the proposed study design.

Methods and analysis

This protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines and is summarized in Table 1.

Design and setting

The study is a dual-centre open-label pilot RCT. It is an interventional study with two arms—protein supplementation and standard nutrition. The study is designed with a reasonable sample size to determine feasibility, study procedures are embedded into routine clinical care and will be executed by clinical personnel. Aside from the study intervention, the clinical diagnosis and management of study participants will be at the discretion of the PICU clinicians.

Clinical research coordinators (CRCs) (Monday – Friday) or study team members (Weekends & Public Holiday) will screen all children daily and maintain screening logs, including reasons for exclusion and reasons why parents of eligible children were not approached for consent.

Study sites and period

This pilot RCT will be conducted in the PICUs at KK Women's and Children's Hospital and the National University Hospital Singapore, two tertiary university affiliated pediatric centres in Singapore. The two centres have different existing nutrition practices, and performing the study procedures in these two centres will make the results more generalizable beyond a few centres with specialized nutritional teams. Should this pilot study be successful, a larger trial will be planned with involvement of other PICUs within the Pediatric Acute & Critical Care Medicine Asian Network (PACCMAN).

Study participants

Children admitted to the PICUs at high risk of protein malnutrition and who are anticipated to remain in the PICU long enough to benefit from protein supplementation will be considered for enrolment. Eligible children may be enrolled in this trial within 48 hours of starting feeds, provided feeding is started within the first 7 days of PICU admission. We chose to limit enrolment based on timing of feeds commencement because we hypothesize that early rather than delayed protein supplementation is important in modulating clinical outcomes. It is anticipated that children need to be exposed to the intervention for 5-7 days to accrue any potential benefit or to experience potential harms. The inclusion and exclusion criteria are summarized in Table 2.

Patient and Public Involvement

Patients and the public were not involved in the design of this protocol.

Risks, adverse events and consent

The potential for adverse events (AE) resulting from the proposed protein supplementation is expected to be minimal as the amount is within the current recommendations of major guidelines, albeit based on low-quality evidence. (11) Additionally, the design of this trial will seek to protect participants from harm by careful participant selection and appropriate

monitoring. Through the exclusion criteria, we will be excluding children at highest risk for adverse effects. Extensive monitoring within the PICU will allow detection and treatment of any adverse effects that do occur.

Monitoring and reporting of AEs and serious adverse events (SAEs) will be carried out in accordance with good clinical practice guidelines. Critically ill patients are at high risk of SAE and the usual approach of reporting all SAEs to the respective ethical boards would result in large numbers of reports of events not related to the trial intervention, but rather reflect the underlying disease process or expected complications of critical illness. (12) The most likely AEs associated with the study interventions are the development of feeding intolerance and diarrhoea, both of which are captured as outcomes and thus not reported as serious adverse events. Only SAEs that might reasonably be judged a consequence of participation in the trial and are judged by the investigators as not due to the underlying disease or expected complications of critical illness will be reported to the ethical boards. SAEs reporting will be performed within 24 hours to the reviewing ethical board and the data and safety monitoring board (DSMB).

Participants may be withdrawn from the study at any time due to an AE or SAE. These will be followed-up by the study team until the clinical outcome from the AE is determined. Examples include:

Prolonged feeding intolerance: Tolerating less than 50% of feeds prescribed over the period of ≥ 5 days
Development of acute kidney injury (according to KDIGO criteria) requiring dialysis(13)
Suspicion of enterocolitis
Significant gastrointestinal bleed requiring consideration for procedural intervention
On request by treating primary physicians

Research staff will approach the child's parents or legal guardians for consent of their child to participate in this trial. Potential benefits and risks will be written in the informed consent document. Patients and parents will be informed of the purpose, intervention, benefits and possible risks of the study. Whenever possible, assent will be obtained from children above 6 years old when the patient has emerged from a critical illness state.

Randomization, allocation concealment and blinding

Participants will be randomized to protein supplementation or standard care in a 1:1 ratio in undisclosed block size by sealed opaque envelope using a computer generated, centrally prepared allocation schedule by the study's biostatistics team. This randomization will be stratified according to centre. Clinical research coordinators or study team members will approach eligible patients for consent. Once consented, study team members will assign participants to allocated interventions.

All clinicians, bedside staff, and research staff involved in clinical management of the participants, parents and guardians will be unblinded to the treatment allocation.

Study interventions

This trial is an interventional study with two arms. Participants will be randomized to enteral protein supplementation or no enteral protein supplementation (i.e. standard nutrition care). For both trial arms, participants will be provided with enteral nutrition (EN) as per standard of care in each centre. General principles of the provision of EN should include a stepwise

progression of feeding volume individualized to the patient's weight, age and clinical status with close monitoring of tolerance by the nurses. Provision of EN values will be verified against nutritional requirements calculated by the dietitian. Children in both arms of the study will be fed so that the final feed volume will meet target energy requirements as calculated using the Schofield equation, with adjustments according to dietitian's assessment.(11) A 10% variation in energy intake per day will be allowed in both arms for ease of preparation of feeds. This variation is acceptable as commonly accepted definitions of overfeeding include a lower limit of 110-120% of caloric requirement. (14) Initiation of parenteral nutrition (PN) as part of primary team's management will be allowed in both arms of the study. Should feeding interruptions occur within either group due to clinical care, these will not be considered protocol violations.

Protein supplementation to achieve a final goal of 1.5 g/kg/day of protein on full feeds will be administered enterally and continue for a total of 7 days from study enrolment or until PICU discharge, whichever occurs earlier. Protein supplementation will consist of 100% whey protein isolate (Beneprotein ®, Nestle, Vevey, Switzerland). If a recovering patient is able to take per oral solid feeds during the study intervention period, the intervention will be suspended due to the variability of oral dietary intake and difficulty in estimating protein and energy intake. If, however, a recovering patient no longer requires enteral feeding but continues to take per oral liquid/milk feeds, the intervention will continue until the stipulated timeframe. The study intervention will be stopped if the attending medical team believes withdrawal of the participant from the study is critical. At this stage, the treating team can follow their usual practice with respect to nutrition provision.

Data collection and management

Data collected will include baseline characteristics, PICU support therapies and detailed nutrition data (Table 1). The collection of nutrition data is a key component of this pilot study. Data pertaining to nutritional intakes of the participants will be collected. These include the following:

- Independent dietician estimation of energy (e.g., Schofield equation) and protein requirement
- EN volume delivered and corresponding calories and protein received
- Highest and lowest glucose levels in the first 24 hours and first week of PICU admission
- Daily fluid balance and electrolytes
- Any PN orders (within the first 7 days of study) and amount of calories and proteins given to the patient will be also be collected

Data will be extracted from electronic medical records by research staff who will enter the data directly into a secure web application (REDCap) hosted by Singapore Clinical Research Institute (SCRI).(15) The database will include both range checks and logic checks and will alert users to any missing data. The database will be stored at SCRI on a secure, firewall protected server with regular backups. Data can be entered by designated and trained users or survey respondents from any computer with an internet connection. User accounts incorporate electronic signatures comprised of a username and password. An audit trail is generated for all activity within each REDCap project.

Study outcomes

The pilot trial will focus on four primary feasibility outcomes and secondary clinical outcomes (Table 3). Change in muscle size and anthropometry will be measured in relation to

measurements performed at PICU admission, or the first measurement. Change in functional status will be measured in relation to the pre-morbid function, which will be obtained from caregiver reports.

Sample size and interim analysis

Based on our preliminary data, we expect to have approximately 48 patients per year meet eligibility criteria for our pilot study. Our projection is that we will have 144 patients over the period of 3 years (48 x 3.0). Assuming a conservative consent rate of 55%, we anticipate 79 patients with BMI z-score ≤ -2 with 39 in each arm. Accounting for a mortality rate of 10%, we anticipate 35 patients per arm for analysis. Sample sizes of n=35 per arm will also have at least 90% probability of correctly selecting a superior arm by 0.33 SD (small-to-moderate effect size) compared to the other arm based on clinical outcomes. (16, 17) To ensure we are able to assess the feasibility and test study procedures and infrastructure at each site, we will aim to enrol at least 15 participants per centre.

Should recruitment be slow and challenging, the study team will meet and decide on the best method in increase enrolment. Some *a priori* strategies that we will consider include (but not limited to) changing the criteria to include:

- Children on non-invasive ventilation or respiratory distress, and requiring any form tube-feeding
- Children with BMI < -1 on PICU admission

Statistical analysis

All analyses will be performed using an intention-to-treat principle. There will be no interim efficacy analyses for this pilot trial. If, after the completion of the pilot trial, the study team determines that there are no important changes to the inclusion and exclusion criteria, the results will not be unblinded for the clinical outcomes of the pilot trial (Figure 1). Instead, we will report the feasibility outcomes, present the clinical outcomes as a single cohort, and consider the pilot trial to be an internal pilot, meaning that we will include the pilot trial patients in the larger RCT. If the study team determines a large trial is not feasible or if including the pilot trial patients in the larger RCT is inappropriate, the clinical outcomes and group comparisons will be reported so that the trial can be included in future meta-analyses. We will use the CONSORT guidelines for reporting.(18, 19)

Feasibility Analysis

Feasibility will be demonstrated by (1) achieving recruitment targets (effective screening, timely enrolment and satisfactory participant accrual), (2) demonstrating at least 80% regimen compliance to allocated groups, and (3) demonstrating safety of the intervention. For the feasibility outcomes we will report the proportions of children meeting each criterion and the associated 95% confidence intervals. We will also compare total protein received by participants in the groups. The number of participants who consented (or not consented) and completed (or discontinued early) the study and the reasons for non-consent/ discontinuation will be summarized using counts and percentages. Demographic and baseline characteristics will also be summarized using descriptive statistics. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of protein supplementation.

Clinical Outcome Analysis

PICU and hospital mortality rate in each arm and differences between the protein supplementation and standard of care arms will be presented with exact 95% confidence intervals. Similarly, means of continuous outcomes (PICU LOS, hospital LOS, and MV

duration) will be presented along with corresponding 95% confidence intervals. Depending on assumption viability, a log-transformed mean or median values will be presented with highly skewed outcomes. Patients who die will be excluded from the analysis of PICU LOS, hospital LOS and MV duration. Further analysis will be performed accounting impact of potential demographic and baseline values of clinical covariates and adjusted difference between the study arms will be presented along with 95% confidence intervals.

Differences in total hospital LOS, PICU stay and duration of MV observed in the protein supplementation group relative to the standard care group will be assessed by subgroup according to illness severity level as characterized by PIM3 scores.

Handling of missing data
Baseline characteristics, PICU support therapies, nutrition and outcome data will be recorded in the electronic medical record system. Therefore, data is very unlikely to be missing. Trained clinical research coordinators will enter data into the REDcap system which will have both range checks and logic checks and alert users to any missing data. If data are still missing, no imputation will be done.

Data safety monitoring
An independent Data Safety Monitoring Committee (DSMC) comprised of three members with experience and expertise in methods, statistics and critical care collectively will monitor the progress and safety of the trial. The DSMC will meet and review the available data when 30% of randomized patients (total of 20 patients or at least 10 in each arm) have completed one month of follow-up. Additional meetings may be held at the discretion of the Chair of the DSMC. The committee will receive SAE reports as they occur. All data will be presented to the DSMC tabulated by intervention group, but the members will remain blinded to the actual group assignment. The committee will review SAEs and centre performance (enrolment, data quality and protocol adherence) and any pertinent external data such as newly published studies or other potentially relevant safety information. They may recommend early termination of the trial if there are SAEs associated with the trial intervention, but no formal stopping rules will be used: this decision will be based on clinical judgment of the DSMC. The DSMC will keep all trial data, committee deliberations and meeting minutes confidential until the end of the trial.

Discussion

Though primarily designed to assess feasibility, this study will be the first RCT investigating the benefits/risks of protein supplementation in addition to standard nutrition in critically ill children. Continuation of this pilot trial into the definitive multicentre RCT will address an important scientific hypothesis—does early enteral protein supplementation of 1.5g/kg/day improve clinical, functional and nutritional outcomes in critically ill children. Numerous prior observational studies with similar aims (1, 5) were inadequately controlled for important selection biases, that is, sicker patients selectively received less nutrition (including less protein). As such, drawing a conclusion that higher nutrition (including higher protein) intake is associated with improved outcomes is inherently biased. A randomized design, such as the proposed study, is the only way to control for such bias.

In critical illness (e.g., sepsis, major surgery), changes in endocrine-metabolic responses lead to an imbalance in protein synthesis and degradation.(20) A negative protein balance is

associated with immunosuppression, poor wound healing, loss of lean muscle mass and a delay in the recovery process.(21) Muscle catabolism is inevitable in acute illness and its intensity depends on the severity of illness. (20) With exogenous nutritional protein and sufficient energy intake, it is postulated that lean muscle mass can be diverted away from oxidative metabolic pathways and preserved. (22) It is, however, unknown what constitutes the optimal amount of protein required to minimize loss of lean muscle mass and the optimal timing of administration in relation to critical illness. Prevailing data from adult studies demonstrate benefits (improved muscle mass (23), reduced mortality (24, 25)), as well as, harm (muscle wasting (8), increased mortality (26)) associated with protein intake in critical illness. These adult data cannot be extrapolated to children, whose protein and energy requirements are inherently different. (27)

There are currently several recommendations for protein requirements during critical illness. The 2018 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommends 1.3g/kg/day protein equivalents be delivered in critically ill adults.(28) In contrast, the American Society for Parenteral and Enteral Nutrition (ASPEN) in conjunction with the Society of Critical Care Medicine (SCCM) 2016 guidelines for critically ill adults recommends 1.2-2.0g/kg/day of protein intake.(29) In critically ill children the recommended protein requirement according to the ASPEN 2017 guidelines was 1.5g/kg/day, acknowledging that the optimal protein intake required to attain a positive protein balance may be much higher than this minimum threshold.(30) It was also suggested that provision of protein early in the course of critical illness was desirable to promote positive nitrogen balance. (30) The provision of 1.5g/kg/day of enteral protein in our intervention arm is based on these guidelines and on translational studies indicating that at least 1.5g/kg/day of protein was required to equilibrate nitrogen and energy balances in critically ill children. (31, 32) Thus far, there have been no clinical trials supporting these recommendations. Moreover, we chose to focus on nutritionally high-risk patients (BMW z-score $\leq$ -2) who have the greatest potential to benefit from nutritional therapy. (33)

Despite the benefits of a randomized design, our pilot RCT may be susceptible to some potential bias. The pragmatic design of this study allows the managing clinical team (including nurses and physicians) and investigators in charge of enrolling participants to be unblinded to the intervention. However, blinding will be maintained for all other research staff, such as statisticians. Non-protein calories which may in itself indirectly affect protein catabolism (34) and clinical outcomes (25, 35), will be recorded and analysed but will not be strictly controlled. Lastly, sedation practices, physical activity (36, 37) and early rehabilitation (38) (which are challenging to control) may interact with nutritional therapy to affect clinical, nutritional and functional outcomes measured in this study. These factors will be taken into account during data analysis.

Trial status

This trial has obtained ethics approval and clinical trial registration. Patient recruitment is anticipated to begin on 4th January 2021 and completed on 3rd January 2023. Follow-up will be completed by 30th June 2023.

Acknowledgments

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Contributors

JHL, JJMW, JSMO, CSO, JCA and FKC conceived and designed this study. JHL, JCA and MG designed the statistical plan. JHL, JJMW, JSMO and CSO obtained permission from the ethics committees. JHL, JJMW, JSMO, CSO, JCA, FKC, LJF, RT, JKBL and PFP will carry out this trial. JHL, JJMW, CSO and JCA drafted this manuscript. JSMO, FKC, LJF, RT, JKBL and PFP carefully reviewed the manuscript; and all authors read and approved the final manuscript.

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Ethics approval
This study has been reviewed and approved by the SingHealth Centralised Institutional Review Board (CIRB) and National Healthcare Group Domain Specific Review Board (DSRB). The study will be conducted in adherence to Singapore Good Clinical Practice (GCP) guidelines. This trial does not need approval with the Health Science Authority of Singapore. This study is registred with clinicaltrials.gov (NCT04565613).

Competing interest
Nil

Patient consent for publication
Not required

Word count: 3533 (4000)

Table 1: SPIRIT schedule of enrolment, interventions, and assessments

Study phase	PICU admission	Screening phase		Treatment phase								Follow-up phase		
Scheduled timeline (from the day of randomization)		**D -7 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**EOT***	**PICU discharge**	**Hospital discharge**	**6 months**
Inclusion/exclusion criteria		•												
Demographics[a]		•												
Medical/surgical history		•												
Informed consent		•												
Randomization			•											
Allocation			•											
Calculation of protein supplementation			•											
Investigational product administration														
Protein Supplementation + Standard nutrition*				•	•	•	•	•	•	•				
Standard nutrition (control arm)				•	•	•	•	•	•	•				
Data collection														
Feasibility data[b]		•	•	•										
Clinical data[c]		•	•	•	•	•	•	•	•	•		•	•	
Nutrition data[d]				•	•	•	•	•	•	•				
Laboratory assessment														
Blood sample: blood sugar[e]				•			•**							
Blood sample: renal panel[f]				•			•**							
Outcome assessments														
Clinical outcomes[g]												•	•	•
Safety assessments														
Physical examination[h]				•	•	•	•	•	•	•				
Vital signs[i]				•	•	•	•	•	•	•				
Adverse and serious events collection[j]				•	•	•	•	•	•	•	•	•	•	•
Muscle mass and functional status assessments														
Muscle US[k]					•					•		•	•	•
Functional Status Scale Score					•					•		•	•	•
PEDI-CAT					•					•		•	•	•

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EOT: end of treatment
PICU: Pediatric Intensive Care Unit
US: Ultrasound
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test.

*Patients will be considered to have reached EOT based on the following:
Complete 7 days of protein supplementation, PICU discharge, the patient has recovered enough to start oral solid feeds, the attending medical team withdraws the patient from the study, death

**Results of blood glucose and renal panel throughout the week, done for clinical indications, will be recorded. If none are clinically indicated, a minimum of 2 measurements will be done for the purposes of this study

[a]Demographics: Age, weight, height, midarm circumference
[b]Feasibility data: proportion of eligible patients approached for consented, number of patients receiving intervention by 72 hours of enrolment, adherence to intervention protocol
[c]Clinical data: baseline characteristics, severity score (Pediatric Index of Mortality 3), PICU support therapies
[d]Nutrition data: nutritional requirements will be calculated (Schofield for calories and 1.5g/kg/day for protein), nutrition prescribed and delivered (calories, protein, carbohydrate, fat, micronutrients) for enteral and parenteral nutrition, fluid input and output.
[e]Blood sugar: measurement from bedside finger-prick glucose meter or plasma glucose, on at least three occasions
[f]Renal panel: serum urea, sodium, potassium, chloride, bicarbonate and creatinine
[g]Clinical outcomes: PICU mortality, PICU length of stay, hospital length of stay, duration of ventilation
[h]Physical examination: evaluation of the cardiovascular, respiratory, abdominal, genitourinary, neurological and musculoskeletal system
[i]Vital signs: heart rate, systolic and diastolic blood pressure, body temperature, respiratory rate, oxygen saturation and pain score
[j]Adverse and serious adverse events includes but not limited to prolonged feeding intolerance (tolerating <50% feeds for ≥ 5days, development of acute kidney injury requiring dialysis, suspicion of enterocolitis, gastrointestinal hemorrhage requiring procedural intervention. If the adverse/serious adverse event is related to the investigational product, participants may be withdrawn and followed up by the study team until clinical outcome of the adverse event is determined
[k]Muscle ultrasound: baseline measurement of rectus femoris cross-sectional area and diaphragm thickness will be taken within 72 hours of enrolment

Table 2: Inclusion and exclusion criteria

Inclusion criteria:	Children (28 days to 18 years of age) BMI z-score ≤ -2 on PICU admission Invasive MV beginning within 24-48 hours of PICU admission and anticipated to continue for ≥ 48 hours Enteral nutrition support for feeding (e.g., orogastric, nasogastric, gastrostomy, nasojejunal, orojejunal)
Exclusion criteria:	Contraindications to enteral nutrition (e.g., gut hemorrhage, post-gastrointestinal surgery, necrotizing enterocolitis, ischemic bowel etc.) Cow's milk protein allergy Anorexia nervosa and other eating disorders Premature infants (corrected gestational age of < 44 weeks) Total parenteral nutrition Extra-corporeal membrane oxygenation Conditions requiring significant fluid restriction (≤75% of maintenance fluids) (e.g., post cardiac surgery) Progressive neuromuscular disease (e.g., spinal muscular atrophy, Duchenne or other muscular dystrophy, multiple sclerosis, amyotrophic lateral sclerosis) Medical conditions where increased or decreased protein intake is required, including acute kidney injury (stage 3 KDIGO criteria), chronic kidney disease (stage 4 and 5), inborn errors of metabolism, fulminant liver failure, severe burn injury Patients who are not expected to survive this PICU admission (e.g., palliative care, do-not-resuscitate orders, limitation of care orders) Previously enrolled in this trial Enrolled in a potentially confounding trial

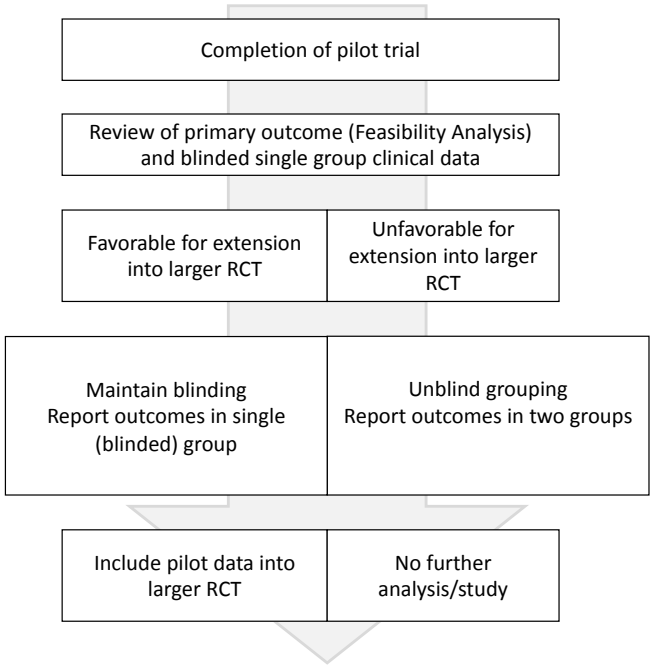
BMI: Body mass index
PICU: Pediatric Intensive Care Unit
MV: Mechanical ventilation
KDIGO: Kidney Disease Improving Global Outcomes

Table 3: Study outcomes

Primary feasibility outcomes	Proportion of eligible patients approached for consent Proportion of participants receiving their first protein supplementation within 72 hours of enrolment Participant accrual, defined as an average monthly enrolment of at least one participant per centre Protocol adherence, defined as >80% of protein target administered according to the protocol in the intervention arm
Secondary clinical outcomes	PICU mortality PICU LOS Hospital LOS MV duration Development of AEs including feeding intolerance, diarrhoea, GI bleeding, and treatment used for GI bleeding Change in muscle size (e.g., ultrasound guided cross-sectional area of the rectus femoris, diaphragm thickness) during PICU stay, at PICU discharge, hospital discharge and 6-months later Change in anthropometric measurements (height, weight, BMI) at PICU discharge, hospital discharge and 6-months later Change in functional status (PEDI-CAT score, FSS score, hand-grip strength and 6-minute walk test) at hospital discharge and 6-months later

PICU: Pediatric Intensive Care Unit
LOS: Length of Stay
MV: Mechanical ventilation
AE: Adverse Effects
GI: Gastrointestinal
BMI: Body mass index
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test
FSS: Functional Status Score

Figure 1: Flowchart for analytical approach of pilot trial



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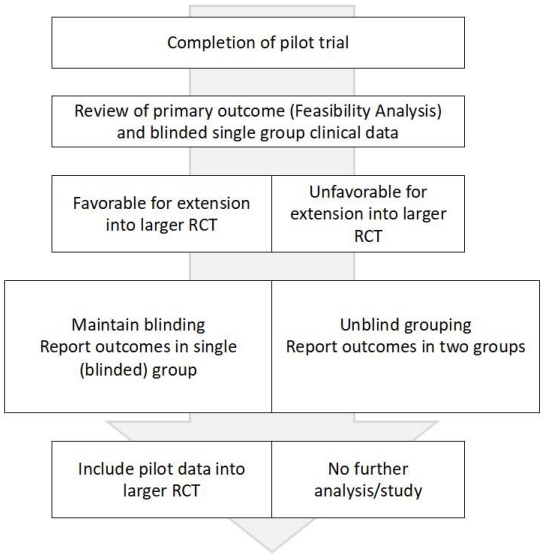

Figure 1: Flowchart for analytical approach of pilot trial

352x198mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	10
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	10
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	3
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	4

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	4
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	6

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	6
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	8
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	2
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	2
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Protein Supplementation versus Standard Feeds in Underweight Critically Ill Children: A Dual-Centre Randomized Controlled Pilot Trial

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Protein Supplementation versus Standard Feeds in Underweight Critically Ill Children: A Dual-Centre Randomized Controlled Pilot Trial

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Abstract

Introduction

Protein-energy malnutrition, increased catabolism in critical illness and inadequate nutritional support leads to loss of lean body mass with muscle wasting and delayed recovery. However, there remains clinical equipoise regarding the risks and benefits of protein supplementation. This pilot trial will determine the feasibility of performing a larger multicentre trial to determine if a strategy of protein supplementation in critically ill children with body mass index (BMI) z-score ≤ -2 is superior to standard enteral nutrition in reducing the length of stay in the paediatric intensive care unit (PICU).

Methods and analysis

This is a randomized controlled trial of 70 children in two PICUs in Singapore. Children with BMI z-score ≤ -2 on PICU admission, who are expected to require invasive mechanical ventilation for more than 48 hours, will be randomized (1:1 allocation) to protein supplementation of ≥1.5g/kg/day in addition to standard nutrition, or standard nutrition alone for 7 days after enrolment or until PICU discharge, whichever is earlier. Feasibility outcomes for the trial include effective screening, satisfactory enrolment rate, timely protocol implementation (within first 72 hours) and protocol adherence. Secondary outcomes include mortality, PICU length of stay, muscle mass, anthropometric measurements and functional outcomes.

Ethics and dissemination

The trial protocol was approved by the institutional review board of both participating centres (Singhealth Centralised Institutional Review Board and National Healthcare Group Domain Specific Review Board) under the reference number 2020/2742. Findings of the trial will be disseminated through peer-reviewed journals and scientific conferences.

Trial registration number: NCT04565613

Strengths and limitations of this study

- To our knowledge, this is the first randomised controlled trial applying enteral protein supplementation to critically ill children
- There is no consensus on the optimal dose for protein intake during pediatric critical illness. Following recommendations of the American Society of Parenteral and Enteral Nutrition (ASPEN), this study will administer 1.5g/kg/day of protein to critically ill children. We chose to focus our study on nutritionally high-risk patients (BMI z-score ≤ -2) who have the greatest potential to benefit from nutritional therapy.
- As the distribution of malnourished children (as defined by a BMI z-score ≤ -2) and PICU support/therapies are variable geographically, the study will employ randomization by centre to achieve balance in treatment allocation within each centre and account for centre-specific effects in the analysis.

Introduction

Background and rationale

Pediatric malnutrition is defined as an imbalance between nutrient requirement and intake resulting in cumulative deficits of energy, protein, or micronutrients.(1) Pediatric malnutrition is pervasive in paediatric intensive care unit (PICU) patients with a prevalence of approximately 18-24% across the world (2-4). Protein malnutrition is caused by insufficient intake or proper utilization of energy and protein leading to increased protein catabolism and was shown to occur in up to 40% of critically ill children.(5-7) Increased catabolism of protein is likely attributable to a combination of various factors including critical illness inflammation, immobility and inadequate nutrition support.(8) Inadequate nutritional provision has been reported in several PICU studies, with reported rates of protein inadequacy ranging from 37 to 87%. (9-11) Inadequate protein intake is associated with poor clinical outcomes in critically ill children. In a large, multicentre cohort study, protein intake ≤ 60% of the prescribed amount was associated with greater odds of mortality compared to those that received >60% of prescribed protein. (10) This was also demonstrated in critically ill children with acute respiratory distress syndrome (ARDS) requiring mechanical ventilation (MV) where children with protein intake of at least 1.5g/kg/day by day 3 of PICU stay had lower risk of mortality.(9) Other concerns of inadequate protein include the loss of lean body mass with muscle wasting and subsequent functional disability, delayed MV weaning, prolonged hospital stay and increased mortality. (12, 13)

There is marked heterogeneity of patients admitted to the PICU. One subset of patients shown to be at high risk of increased morbidity and mortality are those who are underweight on PICU admission. (2, 14) It is hypothesized that children who are underweight have reduced body stores and are thus at greater risk of nutritional decline in the event of nutrient inadequacy (14). As such, a targeted approach of protein supplementation in this particular group of patients can potentially lead to improved clinical outcomes. Thus far, there are no trials evaluating the benefits/risks of supplemental enteral protein administration to critically ill children, highlighting the presence of clinical equipoise.

Due to the inherent challenges of completing randomized controlled trials (RCTs) in pediatric critical care, a rigorous pilot RCT is crucial to evaluate the feasibility of a large RCT. A pilot trial may prevent pursuit of a trial that is ultimately not feasible. This pilot trial is a step towards the large trial needed to provide high-quality, compelling evidence required to develop guidelines for nutrition care in the PICU.

Objectives

The objectives of this pilot trial are to determine the feasibility, efficacy and safety of conducting a large multicentre RCT on protein supplementation in critically ill children. Feasibility related objectives include determination of the proportion of eligible patients approached for consent, likelihood of participants receiving their first protein supplementation within 72 hours of enrolment, participant accrual and protocol adherence. Since this is a pilot trial, efficacy objectives are secondary and will include a reduction in PICU mortality, length of stay, and an improvement in muscle mass, anthropometric measures and functional status at pre-determined follow-up intervals. Safety objectives include surveillance for adverse effects of protein supplementation—including feed intolerance, acute kidney injury, enterocolitis and other gastrointestinal related complications. This pilot trial will also refine inclusion and exclusion criteria, test study procedures,

streamline data collection, and assess parental and physician acceptance of the proposed study design.

Methods and analysis

This protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines and is summarized in Table 1.

Design and setting

The study is a dual-centre open-label pilot RCT. It is an interventional study with two arms—protein supplementation and standard nutrition. The study is designed with a reasonable sample size to determine feasibility, study procedures are embedded into routine clinical care and will be executed by clinical personnel. Aside from the study intervention, the clinical diagnosis and management of study participants will be at the discretion of the PICU clinicians.

Clinical research coordinators (CRCs) (Monday – Friday) or study team members (Weekends & Public Holiday) will screen all children daily and maintain screening logs, including reasons for exclusion and reasons why parents of eligible children were not approached for consent.

Study sites and period

This pilot RCT will be conducted in the PICUs at KK Women's and Children's Hospital and the National University Hospital Singapore, two tertiary university affiliated pediatric centres in Singapore. The two centres have different existing nutrition practices, and performing the study procedures in these two centres will make the results more generalizable beyond a few centres with specialized nutritional teams. Should this pilot study be successful, a larger trial will be planned with involvement of other PICUs within the Pediatric Acute & Critical Care Medicine Asian Network (PACCMAN).

Study participants

Children admitted to the PICUs BMI z-score ≤ -2 on PICU admission and who are anticipated to remain in the PICU long enough to benefit from protein supplementation will be considered for enrolment. Eligible children may be enrolled in this trial within 48 hours of starting feeds, provided feeding is started within the first 7 days of PICU admission. We chose to limit enrolment based on timing of feeds commencement because we hypothesize that early rather than delayed protein supplementation is important in modulating clinical outcomes. It is anticipated that children need to be exposed to the intervention for 5-7 days to accrue any potential benefit or to experience potential harms. The inclusion and exclusion criteria are summarized in Table 2. Children enrolled in a potentially confounding trial with biological interaction affecting outcome measures or adverse events will be excluded. However, if there are no identifiable biological interactions, the study team may consider co-enrolment in both trials.

Patient and Public Involvement

Patients and the public were not involved in the design of this protocol.

Risks, adverse events and consent

The potential for adverse events (AE) resulting from the proposed protein supplementation is expected to be minimal as the amount is within the current recommendations of major guidelines, albeit based on low-quality evidence. (15) Additionally, the design of this trial will seek to protect participants from harm by careful participant selection and appropriate monitoring. Through the exclusion criteria, we will be excluding children at highest risk for adverse effects. Extensive monitoring within the PICU will allow detection and treatment of any adverse effects that do occur including refeeding syndrome.

Monitoring and reporting of AEs and serious adverse events (SAEs) will be carried out in accordance with good clinical practice guidelines. Critically ill patients are at high risk of SAE and the usual approach of reporting all SAEs to the respective ethical boards would result in large numbers of reports of events not related to the trial intervention, but rather reflect the underlying disease process or expected complications of critical illness. (16) The most likely AEs associated with the study interventions are the development of feeding intolerance and diarrhoea, both of which are captured as outcomes and thus not reported as serious adverse events. Only SAEs that might reasonably be judged a consequence of participation in the trial and are judged by the investigators as not due to the underlying disease or expected complications of critical illness will be reported to the ethical boards. SAEs reporting will be performed within 24 hours to the reviewing ethical board and the data and safety monitoring board (DSMB).

Participants may be withdrawn from the study at any time due to an AE or SAE. These will be followed-up by the study team until the clinical outcome from the AE is determined. Examples include:

Prolonged feeding intolerance: Tolerating less than 50% of feeds prescribed over the period of ≥ 5 days
Development of acute kidney injury (according to KDIGO criteria) requiring dialysis(17)
Suspicion of enterocolitis
Significant gastrointestinal bleed requiring consideration for procedural intervention
On request by treating primary physicians

Research staff will approach the child's parents or legal guardians for consent of their child to participate in this trial. Potential benefits and risks will be written in the informed consent document. Patients and parents will be informed of the purpose, intervention, benefits and possible risks of the study. Whenever possible, assent will be obtained from children above 6 years old when the patient has emerged from a critical illness state.

Randomization, allocation concealment and blinding

Participants will be randomized to protein supplementation or standard care in a 1:1 ratio in undisclosed block size by sealed opaque envelope using a computer generated, centrally prepared allocation schedule by the study's biostatistics team. This randomization will be stratified according to centre. Clinical research coordinators or study team members will approach eligible patients for consent**.** Only after consented, will the study team members assign participants to allocated interventions – a model of prior consent will be adopted for this study.

All clinicians, bedside staff, and research staff involved in clinical management of the participants, parents and guardians will be unblinded to the treatment allocation.

Study interventions

This trial is an interventional study with two arms. Participants will be randomized to enteral protein supplementation or no enteral protein supplementation (i.e. standard nutrition care). For both trial arms, participants will be provided with enteral nutrition (EN) as per standard of care in each centre. General principles of the provision of EN using polymeric formula should include a stepwise progression of feeding volume individualized to the patient's weight, age and clinical status with close monitoring of tolerance by the nurses. Provision of EN values will be verified against nutritional requirements calculated by the dietitian. Children in both arms of the study will be fed so that the final feed volume will meet target energy requirements as calculated using the Schofield equation, with adjustments according to dietitian's assessment.(15) A 10% variation in energy intake per day will be allowed in both arms for ease of preparation of feeds.(18) Should feeding interruptions occur within either group due to clinical care, these will not be considered protocol violations.

Protein supplementation will be administered enterally and continue for a total of 7 days from study enrolment or until PICU discharge, whichever occurs earlier. Protein supplementation will consist of 100% whey protein isolate (Beneprotein ®, Nestle, Vevey, Switzerland). Protein supplementation will be provided in divided doses throughout the day and added to the prescribed milk formula feed regime to ensure a total protein intake of 1.5 g/kg/day when full feeds are achieved. For example, a child with a weight of 25kg receiving standard polymeric formula, would have an approximate intake of 40kcal/kg/day and 1.2g/kg/day protein. An additional 7.5g of protein is required, which is approximately equal to 1.25 scoops of protein supplement per day.

If a recovering patient is able to take per oral solid feeds during the study intervention period, the intervention will be suspended due to the variability of oral dietary intake and difficulty in estimating protein and energy intake. If, however, a recovering patient no longer requires enteral feeding but continues to take per oral liquid/milk feeds, the intervention will continue until the stipulated timeframe. The study intervention will be stopped if the attending medical team believes withdrawal of the participant from the study is critical. At this stage, the treating team can follow their usual practice with respect to nutrition provision. Parents may also withdraw their child from the study at any point for any reason - should this occur, only data collected up to the point of withdrawal will be utilized in the analysis.

Data collection and management

Data collected will include baseline characteristics, PICU support therapies and detailed nutrition data (Table 1). The collection of nutrition data is a key component of this pilot study. Data pertaining to nutritional intakes of the participants will be collected. These include the following:

- Independent dietician estimation of energy (e.g., Schofield equation) and protein requirement
- EN volume delivered and corresponding calories and protein received
- Highest and lowest glucose levels in the first 24 hours and first week of PICU admission
- Daily fluid balance and electrolytes

Data will be extracted from electronic medical records by research staff who will enter the data directly into a secure web application (REDCap) hosted by Singapore Clinical Research Institute (SCRI).(19) The database will include both range checks and logic checks and will alert users to any missing data. The database will be stored at SCRI on a secure, firewall protected server with regular backups. Data can be entered by designated and trained users or survey respondents from any computer with an internet connection. User accounts incorporate electronic signatures comprised of a username and password. An audit trail is generated for all activity within each REDCap project.

Study outcomes

The pilot trial will focus on four primary feasibility outcomes and secondary clinical outcomes (Table 3). Change in muscle size and anthropometry will be measured in relation to measurements performed within 24hours of PICU admission as an exploratory outcome. Ultrasonography will be used to visualize and capture muscle changes in critically ill children (Appendix 1) (20). Change in functional status, as defined by the functional status scores (FSS) will be measured in relation to the pre-morbid function, and will be obtained from caregiver reports (Appendix 1) (21).

Sample size and interim analysis

The purpose of this pilot study is to investigate whether protein supplementation has promising efficacy and is worth further investigation. A large randomized study with usual care as the active control would be inappropriate as insufficient evidence of benefit of protein supplementation has yet to be obtained to justify such a study. In circumstances involving uncertainty of benefit and need for parsimony in resource expenditures, a small randomized study invoking the 'selection theory' approach proposed by Simon et al (22, 23) can provide an initial assessment of benefit. In the selection theory approach, the objective is to rank multiple potential treatments and then select those with the best responses for further study. However, our study involves only two treatments—protein supplementation versus standard feeds—which simplifies the approach in a determination of whether protein supplementation is better than standard feeds.

In the absence of any prior rates of clinical outcomes or effect size, our study will allow a response assessment and the potential for demonstrating greater efficacy of protein supplementation versus standard feeds in underweight critically ill children, with high statistical power, using a procedure that circumvents a formal hypothesis test.

Effect size is defined as $\delta = (\mu_1 - \mu_2)/\sigma$, where μ_1 and μ_2 represent clinical endpoint population means for the protein supplementation and standard feeds arms, respectively. In calculating sample size in the context of selection theory, we postulate the conventional underlying null and alternative hypotheses of H_0: $\delta \leq 0$ vs H_1: $\delta > 0$, respectively. In our pilot study, we will target an effect size of $\delta = 0.33$, which is considered a small-to-moderate effect size and often viewed as representing a clinically important difference. (24) If protein supplementation is superior to standard feeds by $\delta \geq 0.33$, we desire to detect this difference with power $\geq 90\%$. However, under a true null hypothesis, we will choose to ignore the type I error rate, and so set $\alpha = 50\%$—equivalent to random chance. Performing the sample size calculation based on a one-sided hypothesis test of two independent means using a two-sample t-test with one-sided $\alpha = 0.50$, a sample size of $n = 35$ per group achieves power = 0.92 to detect an effect size of $\delta = 0.33$. (PASS® commercial software was used to perform the sample size calculation.)

From our preliminary data, we expect to have approximately 48 patients per year meet eligibility criteria for our pilot study. Our projection is that we will see 144 eligible patients over the 3-year recruitment period (3 x 48). Assuming a conservative consent rate of 55%, we anticipate at least 80 patients with BMI z-score ≤ -2 which will provide 40 patients in each study arm. Accounting for a dropout rate of 10-12% due to mortality and other causes would anticipate n = 35 patients per arm completing the study (total N = 70), which for $\delta \geq 0.33$ achieves > 90% probability for demonstrating protein supplementation superiority to standard feeds. To ensure we are able to assess feasibility and test study procedures and infrastructure at each site, we aim to enrol 26 or 27 patients per centre per year (13 or 14 per arm).

It is emphasized that under the selection theory paradigm, the best treatment for further consideration in a subsequent larger trial is selected on the basis of descriptive statistics—in this case, higher mean value. Hence, given an effect size of $\delta \geq 0.33$, the proposed procedure and sample size will ensure a > 90% probability of protein supplementation as the better treatment, demonstrated by a higher mean value, without a formal hypothesis test. A 95% confidence interval will be calculated on the protein supplementation versus standard feeds mean difference for the clinical efficacy variables.

Should recruitment be slow and challenging, the study team will meet and decide on the best method in increase enrolment. Some *a priori* strategies that we will consider include (but not limited to) changing the criteria to include:

- Children on non-invasive ventilation or respiratory distress, and requiring any form tube-feeding
- Children with BMI ≤ -1 on PICU admission

Statistical analysis

All analyses will be performed using an intention-to-treat principle. There will be no interim efficacy analyses for this pilot trial. If, after the completion of the pilot trial, the study team determines that there are no important changes to the inclusion and exclusion criteria, the results will not be unblinded for the clinical outcomes of the pilot trial (Figure 1). Instead, we will report the feasibility outcomes, present the clinical outcomes as a single cohort, and consider the pilot trial to be an internal pilot, meaning that we will include the pilot trial patients in the larger RCT. If the study team determines a large trial is not feasible or if including the pilot trial patients in the larger RCT is inappropriate, the clinical outcomes and group comparisons will be reported so that the trial can be included in future meta-analyses. We will use the CONSORT guidelines for reporting.(25, 26)

Feasibility Analysis

Feasibility will be demonstrated by (1) achieving recruitment targets (effective screening, timely enrolment and satisfactory participant accrual), (2) demonstrating at least 80% regimen compliance to allocated groups, (3) demonstrating safety of the intervention and (4) demonstrating delivery of protein with a separation of at least a 0.5g/kg/day in the intervention and control arms. Effective screening will be achieved if 90% of all PICU admissions are screened within 24hours, timely enrolment will be achieved if 90% of all eligible participants ae enrolled with 48hours of meeting eligibility criteria and satisfactory participant accrual is considered if both centres recruit a total of at least 26 patients per year. For the feasibility outcomes we will report the proportions of children meeting each criterion and the associated 95% confidence intervals. We will also compare total protein received by participants in the groups. The number of participants who consented (or not consented) and completed (or discontinued early) the study and the reasons for non-consent/ discontinuation

will be summarized using counts and percentages. Demographic and baseline characteristics will also be summarized using descriptive statistics. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of protein supplementation.

Clinical Outcome Analysis

PICU and hospital mortality rate in each arm and differences between the protein supplementation and standard of care arms will be presented with exact 95% confidence intervals. Medians of continuous variables [PICU LOS, hospital LOS, MV duration, 28-day ventilator-free days (VFD) and PICU-free days (IFD)] will be presented along with corresponding 95% confidence intervals. LOS and duration endpoints will be compared between treatment groups using a log-rank test in conjunction with Kaplan-Meier survival curves. Patients who die will be censored. If warranted, additional analysis using Cox regression will be performed to adjust for the influence of potential demographic and clinical confounders

Differences in total hospital LOS, PICU stay, duration of MV, VFD and IFD observed in the protein supplementation group relative to the standard care group will be assessed by subgroup according to illness severity level as characterized by PIM3 scores (27, 28). Change in muscle size (e.g., ultrasound guided cross-sectional area of the rectus femoris), anthropometry (height, weight, BMI) and functional status (PEDI-CAT score, FSS score, hand-grip strength and 6-minute walk test) during PICU stay, at PICU discharge, hospital discharge and 6-months later will also be measured as exploratory outcomes.

Handling of missing data

Baseline characteristics, PICU support therapies, nutrition and outcome data will be recorded in the electronic medical record system. Therefore, data is very unlikely to be missing. Trained clinical research coordinators will enter data into the REDcap system which will have both range checks and logic checks and alert users to any missing data. If data are still missing, no imputation will be done.

Trial steering committee

There will be a single steering committee overseeing trial execution over the two participating sites. The committee will consist of the two site-principal investigators, two dietitians, two nursing leads and four study team members representative from both sites. This group will be responsible for each step of the trial process including ensuring consistent screening, reviewing recruitment numbers, deliberating on eligibility of participants and adverse events. The steering committee will meet quarterly to discuss progress of the trial and troubleshoot any problems or delays in the project plan.

Data safety monitoring

An independent Data Safety Monitoring Committee (DSMC) comprised of three members with experience and expertise in methods, statistics and critical care collectively will monitor the progress and safety of the trial. The DSMC will meet and review the available data when 30% of randomized patients (total of 20 patients or at least 10 in each arm) have completed one month of follow-up. Additional meetings may be held at the discretion of the Chair of the DSMC. The committee will receive SAE reports as they occur. All data will be presented to the DSMC tabulated by intervention group, but the members will remain blinded to the actual group assignment. The committee will review SAEs and centre performance (enrolment, data

quality and protocol adherence) and any pertinent external data such as newly published studies or other potentially relevant safety information. They may recommend early termination of the trial if there are SAEs associated with the trial intervention, but no formal stopping rules will be used: this decision will be based on clinical judgment of the DSMC. The DSMC will keep all trial data, committee deliberations and meeting minutes confidential until the end of the trial.

Discussion

Though primarily designed to assess feasibility, this study will be the first RCT investigating the benefits/risks of protein supplementation in addition to standard nutrition in critically ill children. Continuation of this pilot trial into the definitive multicentre RCT will address an important scientific hypothesis—does early enteral protein supplementation of 1.5g/kg/day improve clinical, functional and nutritional outcomes in critically ill children. Numerous prior observational studies with similar aims (5, 9) were inadequately controlled for important selection biases, that is, sicker patients selectively received less nutrition (including less protein). As such, drawing a conclusion that higher nutrition (including higher protein) intake is associated with improved outcomes is inherently biased. A randomized design, such as the proposed study, is the only way to control for such bias.

In critical illness (e.g., sepsis, major surgery), changes in endocrine-metabolic responses lead to an imbalance in protein synthesis and degradation.(29) A negative protein balance is associated with immunosuppression, poor wound healing, loss of lean muscle mass and a delay in the recovery process.(30) Muscle catabolism is inevitable in acute illness and its intensity depends on the severity of illness. (29) With exogenous nutritional protein and sufficient energy intake, it is postulated that lean muscle mass can be diverted away from oxidative metabolic pathways and preserved. (31) It is, however, unknown what constitutes the optimal amount of protein required to minimize loss of lean muscle mass and the optimal timing of administration in relation to critical illness. Prevailing data from adult studies demonstrate benefits (improved muscle mass (32), reduced mortality (33, 34), as well as, harm (muscle wasting (12), increased mortality (35) associated with protein intake in critical illness. These adult data cannot be extrapolated to children, whose protein and energy requirements are inherently different. (36)

There are currently several recommendations for protein requirements during critical illness. The 2018 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommends 1.3g/kg/day protein equivalents be delivered in critically ill adults.(37) In contrast, the American Society for Parenteral and Enteral Nutrition (ASPEN) in conjunction with the Society of Critical Care Medicine (SCCM) 2016 guidelines for critically ill adults recommends 1.2-2.0g/kg/day of protein intake.(38) In critically ill children the recommended protein requirement according to the ASPEN 2017 guidelines was 1.5g/kg/day, acknowledging that the optimal protein intake required to attain a positive protein balance may be much higher than this minimum threshold.(39) It was also suggested that provision of protein early in the course of critical illness was desirable to promote positive nitrogen balance. (39) The provision of 1.5g/kg/day of enteral protein in our intervention arm is based on these guidelines and on translational studies indicating that at least 1.5g/kg/day of protein was required to equilibrate nitrogen and energy balances in critically ill children. (40, 41) It is noteworthy, however, that the PEPaNIC trial (early vs. late parenteral nutrition in critically ill children) comparing nutrition supplementation in the form of early parenteral

nutrition within 24 hours of PICU admission vs. late supplementation with parenteral nutrition after the first week of PICU stay demonstrated a higher rate of new infection, prolonged PICU stay and decreased likelihood of being discharged alive from hospital in the early group. In the PEPaNIC trial, the early group received higher protein intake (approximately 1.5g/kg/day) in the form of an intravenous amino acid solution over the first week of PICU stay (42). There are, however, fundamental differences between the current proposed study and PEPaNIC trial which make direct extrapolation of outcomes inappropriate. Firstly, the PEPaNIC trial included critically ill children "at-risk of malnutrition" [using the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids)], whereas, we chose to focus on established underweight patients (BMW z-score ≤ -2) who have the greatest potential to benefit from nutritional therapy. (43) Secondly, the PEPaNIC trial utilized parenteral nutrition instead of EN which in itself has been associated with infections and other poor outcomes. (44-46) As such, an empirical trial of supplemental enteral protein is warranted and will be informative.

Despite the benefits of a randomized design, our pilot RCT may be susceptible to some potential bias. In this dual center RCT, there is no standardized EN protocol between the two centers. Though routine protein supplementation is not currently practiced in both centers, the variable practice may lead to potential overlap in protein dosing between the intervention and control arms. We recognize this as a limitation but are unable to justify ethically to reduce protein intake of patients to below what standard care provides. In addition, a proportion of patients will be excluded from the study due to safety concerns (exclusion criteria) and this will limit the generalizability of this RCT. The pragmatic design of this study also allows the managing clinical team (including nurses and physicians) and investigators in charge of enrolling participants to be unblinded to the intervention. However, blinding will be maintained for all other research staff, such as statisticians. As indirect calorimetry is not readily available at both sites, energy equations would be used to calculate requirements, which could result in energy over or underfeeding. (47) Non-protein calories which may in itself indirectly affect protein catabolism (48) and clinical outcomes (34, 49), will be recorded and analysed but will not be strictly controlled. Lastly, sedation practices, physical activity (50, 51) and early rehabilitation (52) (which are challenging to control) may interact with nutritional therapy to affect clinical, nutritional and functional outcomes measured in this study.

Trial status

This trial has obtained ethics approval and clinical trial registration. Patient recruitment is anticipated to begin on 4th January 2021 and to complete on 3rd January 2024. Follow-up will be completed by 30th June 2024.

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Contributors

JHL, JJMW, JSMO, CSO, JCA and FKC conceived and designed this study. JHL, JCA and MG designed the statistical plan. JHL, JJMW, JSMO and CSO obtained permission from the ethics committees. JHL, JJMW, JSMO, CSO, JCA, FKC, LJF, RT, JKBL and PFP will carry out this trial. JHL, JJMW, CSO and JCA drafted this manuscript. JSMO, FKC, LJF, RT,

JKBL and PFP carefully reviewed the manuscript; and all authors read and approved the final manuscript.

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Ethics approval
This study has been reviewed and approved by the SingHealth Centralised Institutional Review Board (CIRB) and National Healthcare Group Domain Specific Review Board (DSRB). The study will be conducted in adherence to Singapore Good Clinical Practice (GCP) guidelines. This trial does not need approval with the Health Science Authority of Singapore. This study is registered with clinicaltrials.gov (NCT04565613).

Competing interest
Nil

Patient consent for publication
Not required

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Table 1: SPIRIT schedule of enrolment, interventions, and assessments

Study phase	PICU admission	Screening phase		Treatment phase								Follow-up phase		
Scheduled timeline (from the day of randomization)		**D -7 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**EOT***	**PICU discharge**	**Hospital discharge**	**6 months**
Inclusion/exclusion criteria		•												
Demographics[a]		•												
Medical/surgical history		•												
Informed consent		•												
Randomization			•											
Allocation			•											
Calculation of protein supplementation			•											
Investigational product administration														
Protein Supplementation + Standard nutrition*				•	•	•	•	•	•	•				
Standard nutrition (control arm)				•	•	•	•	•	•	•				
Data collection														
Feasibility data[b]		•	•	•										
Clinical data[c]		•	•	•	•	•	•	•	•	•		•	•	
Nutrition data[d]				•	•	•	•	•	•	•				
Laboratory assessment														
Blood sample: blood sugar[e]				•			•**							
Blood sample: renal panel[f]				•			•**							
Outcome assessments														
Clinical outcomes[g]												•	•	•
Safety assessments														
Physical examination[h]				•	•	•	•	•	•	•				
Vital signs[i]				•	•	•	•	•	•	•				
Adverse and serious events collection[j]				•	•	•	•	•	•	•	•	•	•	•
Muscle mass and functional status														
Muscle US[k]					•					•		•	•	•
Functional Status Scale Score					•					•		•	•	•
PEDI-CAT					•					•		•	•	•

EOT: end of treatment
PICU: Pediatric Intensive Care Unit
US: Ultrasound
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test.

*Patients will be considered to have reached EOT based on the following:
Complete 7 days of protein supplementation, PICU discharge, the patient has recovered enough to start oral solid feeds, the attending medical team withdraws the patient from the study, death

**Results of blood glucose and renal panel throughout the week, done for clinical indications, will be recorded. If none are clinically indicated, a minimum of 2 measurements will be done for the purposes of this study

[a]Demographics: Age, weight, height, midarm circumference
[b]Feasibility data: proportion of eligible patients approached for consented, number of patients receiving intervention by 72 hours of enrolment, adherence to intervention protocol
[c]Clinical data: baseline characteristics, severity score (Pediatric Index of Mortality 3), PICU support therapies
[d]Nutrition data: nutritional requirements will be calculated (Schofield for calories and 1.5g/kg/day for protein), nutrition prescribed and delivered (calories, protein, carbohydrate, fat, micronutrients) for enteral and parenteral nutrition, fluid input and output
[e]Blood sugar: measurement from bedside finger-prick glucose meter or plasma glucose, on at least three occasions
[f]Renal panel: serum urea, sodium, potassium, chloride, bicarbonate and creatinine
[g]Clinical outcomes: PICU mortality, PICU length of stay, hospital length of stay, duration of ventilation
[h]Physical examination: evaluation of the cardiovascular, respiratory, abdominal, genitourinary, neurological and musculoskeletal system
[i]Vital signs: heart rate, systolic and diastolic blood pressure, body temperature, respiratory rate, oxygen saturation and pain score
[j]Adverse and serious adverse events includes but not limited to prolonged feeding intolerance (tolerating <50% feeds for ≥ 5days, development of acute kidney injury requiring dialysis, suspicion of enterocolitis, gastrointestinal hemorrhage requiring procedural intervention. If the adverse/serious adverse event is related to the investigational product, participants may be withdrawn and followed up by the study team until clinical outcome of the adverse event is determined
[k]Muscle ultrasound: baseline measurement of rectus femoris cross-sectional area and diaphragm thickness will be taken within 72 hours of enrolment

Table 2: Inclusion and exclusion criteria

Inclusion criteria:	Children (28 days to 18 years of age) Both elective or emergency admissions BMI z-score ≤ -2 on PICU admission Invasive MV beginning within 48 hours of PICU admission and anticipated to continue for ≥ 48 hours Enteral nutrition support for feeding (e.g., orogastric, nasogastric, gastrostomy, nasojejunal, orojejunal)
Exclusion criteria:	Contraindications to enteral nutrition (e.g., gut hemorrhage, post-gastrointestinal surgery, necrotizing enterocolitis, ischemic bowel etc.) Cow's milk protein allergy Anorexia nervosa and other eating disorders Premature infants (corrected gestational age of < 44 weeks) Parenteral nutrition Extra-corporeal membrane oxygenation Conditions requiring significant fluid restriction (≤75% of maintenance fluids) (e.g., post cardiac surgery) Progressive neuromuscular disease (e.g., spinal muscular atrophy, Duchenne or other muscular dystrophy, multiple sclerosis, amyotrophic lateral sclerosis) Medical conditions where increased or decreased protein intake is required, including acute kidney injury (stage 3 KDIGO criteria), chronic kidney disease (stage 4 and 5), inborn errors of metabolism, fulminant liver failure, severe burn injury Patients who are not expected to survive this PICU admission (e.g., palliative care, do-not-resuscitate orders, limitation of care orders) Previously enrolled in this trial Enrolled in a potentially confounding trial

BMI: Body mass index
PICU: Pediatric Intensive Care Unit
MV: Mechanical ventilation
KDIGO: Kidney Disease Improving Global Outcomes

Table 3: Study outcomes

Primary feasibility outcomes	Proportion of eligible patients approached for consent Proportion of participants receiving their first protein supplementation within 72 hours of enrolment Participant accrual, defined as an average monthly enrolment of at least one participant per centre Protocol adherence, defined as >80% of protein target administered according to the protocol in the intervention arm
Secondary clinical outcomes	PICU mortality PICU LOS 28-day PICU-free days Hospital LOS MV duration 28-day ventilator-free days Development of AEs including feeding intolerance, diarrhoea, GI bleeding, and treatment used for GI bleeding Change in muscle size (e.g., ultrasound guided cross-sectional area of the rectus femoris, diaphragm thickness) during PICU stay, at PICU discharge, hospital discharge and 6-months later Change in anthropometric measurements (height, weight, BMI) at PICU discharge, hospital discharge and 6-months later Change in functional status (PEDI-CAT score, FSS score, hand-grip strength and 6-minute walk test) at hospital discharge and 6-months later

PICU: Pediatric Intensive Care Unit
LOS: Length of Stay
MV: Mechanical ventilation
AE: Adverse Effects
GI: Gastrointestinal
BMI: Body mass index
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test
FSS: Functional Status Score

Figure legends

Figure 1: Flowchart for analytical approach of pilot trial

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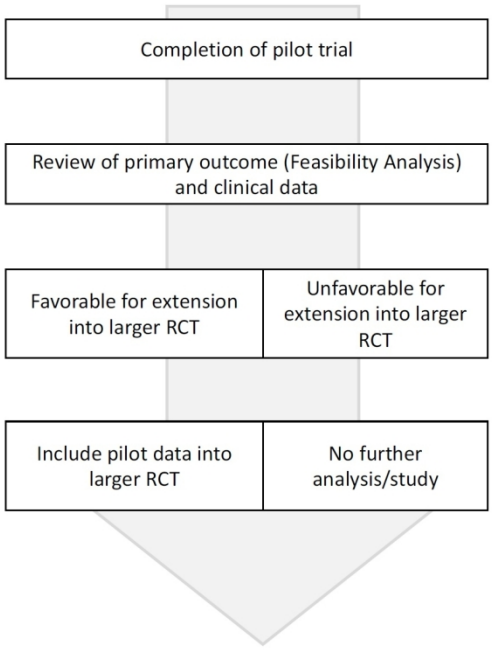

Figure 1: Flowchart for analytical approach of pilot trial

468x345mm (96 x 96 DPI)

Appendix 1: Instructions for ultrasound of rectus femoris muscle

A. Ensure the patient is lying comfortably, with leg extended in neutral position. The head of bed should ideally be inclined at 30 degrees.

B. Locating position
   - Choose the right leg wherever possible. Use the same leg for measurements throughout the study.
   - Locate the base of the iliac crest and the top of the patella. Measure the distance and mark the mid-point (children < 6 years) or 1/3 the distance from the patella (children >6 years).

C. Ultrasound measurement
   1. Use the linear probe with the largest footprint available.
   2. Ensure that the settings are correct. Suggested standardized settings are a frequency of 12.0MHz, Gain of 50 and Dynamic Range (DR) of 95. Ensure that that the time-gain is in the neutral position.
   3. Adjust settings if necessary, between patients. Ideally the image should be as large as possible, while allowing visualization of the skin surface as well as the bone. For each patient, the following settings should remain the same
      i. Depth
      ii. Gain
      iii. Frequency
   4. Create a new exam
      i. Enter in patient ID
      ii. When the rectus femoris can be visualized appropriately, press "freeze" and then save picture.
      iii. For the cross-sectional ultrasound measurement, ensure that there is copious gel and minimal compression of the skin.
      iv. Label image with subject ID, location, scan no. etc. Suggest to record as: SubjectID_location at leg_timepoint of measurement_image number. E.g. ID01_1/2RL_1_3 (this shows subject 1, measured at ½ of right leg, first measurement, image 3.
      v. Press "freeze" again to unfreeze pane, and repeat.
   5. Capture 3 images and save each image. Name each image appropriately.
   6. Export the DICOM images.

D. Measuring the cross-sectional area
   1. Using the appropriate software with DICOM format support (e.g. NIH ImageJ tool), draw the cross-sectional area by tracing the inner echoic edge of the rectus femoris cross sectional area.
   2. Record the cross-sectional area in cm^2

Appendix Table 1. Functional status scale score by Pollack et al. 2009

Domains	Normal (Score = 1)	Mild Dysfunction (Score = 2)	Moderate Dysfunction (Score = 3)	Severe Dysfunction (Score = 4)	Very Severe Dysfunction (Score = 5)
Mental status	Normal sleep/wake periods; appropriate responsiveness	Sleepy but arousable to noise/ touch/ movement and/or periods of social non-responsiveness	Lethargic and/or irritable	Minimal arousal to stimuli (stupor)	Unresponsive, coma, and/or vegetative state
Sensory functioning	Intact hearing and vision and responsive to touch	Suspected hearing or vision loss	Not reactive to auditory stimuli or to visual stimuli	Not reactive to auditory stimuli and to visual stimuli	Abnormal responses to pain or touch
Communication	Appropriate non-crying vocalizations, interactive facial expressiveness, or gestures	Diminished vocalization, facial expression, and/or social responsiveness	Absence of attention getting behavior	No demonstration of discomfort	Absence of communication
Motor functioning	Coordinated body movements, normal muscle control, and awareness of action and reason	1 limb functionally impaired	≥2 limbs functionally impaired	Poor head control	Diffuse spasticity, paralysis, or decerebrate/ decorticate posturing
Feeding	All food taken by mouth with age-appropriate help	Nothing by mouth or need for age-inappropriate help with feeding	Oral and tube feedings	Parenteral nutrition with oral or tube feedings	All parenteral nutrition
Respiratory status	Room air and no artificial support or aids	Oxygen treatment and/or suctioning	Tracheostomy	Continuous positive airway pressure treatment for all or part of the day and/ or mechanical ventilatory support for part of the day	Mechanical ventilator support for all of the day and night

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	10
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	10
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	3
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	4

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	4
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	6

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	6
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	8
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	2
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	2
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

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Protein Supplementation versus Standard Feeds in Underweight Critically Ill Children: A Pilot Dual-Centre Randomized Controlled Trial Protocol

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Protein Supplementation versus Standard Feeds in Underweight Critically Ill Children: A Pilot Dual-Centre Randomized Controlled Trial Protocol

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Abstract

Introduction

Protein-energy malnutrition, increased catabolism in critical illness and inadequate nutritional support leads to loss of lean body mass with muscle wasting and delayed recovery. However, there remains clinical equipoise regarding the risks and benefits of protein supplementation. This pilot trial will determine the feasibility of performing a larger multicentre trial to determine if a strategy of protein supplementation in critically ill children with body mass index (BMI) z-score ≤ -2 is superior to standard enteral nutrition in reducing the length of stay in the paediatric intensive care unit (PICU).

Methods and analysis

This is a randomized controlled trial of 70 children in two PICUs in Singapore. Children with BMI z-score ≤ -2 on PICU admission, who are expected to require invasive mechanical ventilation for more than 48 hours, will be randomized (1:1 allocation) to protein supplementation of ≥1.5g/kg/day in addition to standard nutrition, or standard nutrition alone for 7 days after enrolment or until PICU discharge, whichever is earlier. Feasibility outcomes for the trial include effective screening, satisfactory enrolment rate, timely protocol implementation (within first 72 hours) and protocol adherence. Secondary outcomes include mortality, PICU length of stay, muscle mass, anthropometric measurements and functional outcomes.

Ethics and dissemination

The trial protocol was approved by the institutional review board of both participating centres (Singhealth Centralised Institutional Review Board and National Healthcare Group Domain Specific Review Board) under the reference number 2020/2742. Findings of the trial will be disseminated through peer-reviewed journals and scientific conferences.

Trial registration number: NCT04565613

Strengths and limitations of this study

- To our knowledge, this is the first randomised controlled trial applying enteral protein supplementation to critically ill children
- There is no consensus on the optimal dose for protein intake during pediatric critical illness. Following recommendations of the American Society of Parenteral and Enteral Nutrition (ASPEN), this study will administer 1.5g/kg/day of protein to critically ill children. We chose to focus our study on nutritionally high-risk patients (BMI z-score ≤ -2) who have the greatest potential to benefit from nutritional therapy.
- As the distribution of malnourished children (as defined by a BMI z-score ≤ -2) and PICU support/therapies are variable geographically, the study will employ randomization by centre to achieve balance in treatment allocation within each centre and account for centre-specific effects in the analysis.

Introduction

Background and rationale

Pediatric malnutrition is defined as an imbalance between nutrient requirement and intake resulting in cumulative deficits of energy, protein, or micronutrients.(1) Pediatric malnutrition is pervasive in paediatric intensive care unit (PICU) patients with a prevalence of approximately 18-24% across the world (2-4). Protein malnutrition is caused by insufficient intake or proper utilization of energy and protein leading to increased protein catabolism and was shown to occur in up to 40% of critically ill children.(5-7) Increased catabolism of protein is likely attributable to a combination of various factors including critical illness inflammation, immobility and inadequate nutrition support.(8) Inadequate nutritional provision has been reported in several PICU studies, with reported rates of protein inadequacy ranging from 37 to 87%. (9-11) Inadequate protein intake is associated with poor clinical outcomes in critically ill children. In a large, multicentre cohort study, protein intake ≤ 60% of the prescribed amount was associated with greater odds of mortality compared to those that received >60% of prescribed protein. (10) This was also demonstrated in critically ill children with acute respiratory distress syndrome (ARDS) requiring mechanical ventilation (MV) where children with protein intake of at least 1.5g/kg/day by day 3 of PICU stay had lower risk of mortality.(9) Other concerns of inadequate protein include the loss of lean body mass with muscle wasting and subsequent functional disability, delayed MV weaning, prolonged hospital stay and increased mortality. (12, 13)

There is marked heterogeneity of patients admitted to the PICU. One subset of patients shown to be at high risk of increased morbidity and mortality are those who are underweight on PICU admission. (2, 14) It is hypothesized that children who are underweight have reduced body stores and are thus at greater risk of nutritional decline in the event of nutrient inadequacy (14). As such, a targeted approach of protein supplementation in this particular group of patients can potentially lead to improved clinical outcomes. Thus far, there are no trials evaluating the benefits/risks of supplemental enteral protein administration to critically ill children, highlighting the presence of clinical equipoise.

Due to the inherent challenges of completing randomized controlled trials (RCTs) in pediatric critical care, a rigorous pilot RCT is crucial to evaluate the feasibility of a large RCT. A pilot trial may prevent pursuit of a trial that is ultimately not feasible. This pilot trial is a step towards the large trial needed to provide high-quality, compelling evidence required to develop guidelines for nutrition care in the PICU.

Objectives

The objectives of this pilot trial are to determine the feasibility, efficacy and safety of conducting a large multicentre RCT on protein supplementation in critically ill children. Feasibility related objectives include determination of the proportion of eligible patients approached for consent, likelihood of participants receiving their first protein supplementation within 72 hours of enrolment, participant accrual and protocol adherence. Since this is a pilot trial, efficacy objectives are secondary and will include a reduction in PICU mortality, length of stay, and an improvement in muscle mass, anthropometric measures and functional status at pre-determined follow-up intervals. Safety objectives include surveillance for adverse effects of protein supplementation—including feed intolerance, acute kidney injury, enterocolitis and other gastrointestinal related complications. This pilot trial will also refine inclusion and exclusion criteria, test study procedures,

streamline data collection, and assess parental and physician acceptance of the proposed study design.

Methods and analysis

This protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines and is summarized in Table 1.

Design and setting

The study is a dual-centre open-label pilot RCT. It is an interventional study with two arms—protein supplementation and standard nutrition. The study is designed with a reasonable sample size to determine feasibility, study procedures are embedded into routine clinical care and will be executed by clinical personnel. Aside from the study intervention, the clinical diagnosis and management of study participants will be at the discretion of the PICU clinicians. Dietitians, who are study team members in the two centres, will be involved in the trial design and reviewing the nutrition plan of all trial participants

Clinical research coordinators (CRCs) (Monday – Friday) or study team members (Weekends & Public Holiday) will screen all children daily and maintain screening logs, including reasons for exclusion and reasons why parents of eligible children were not approached for consent.

Study sites and period

This pilot RCT will be conducted in the PICUs at KK Women's and Children's Hospital and the National University Hospital Singapore, two tertiary university affiliated pediatric centres in Singapore. The two centres have different existing nutrition practices, and performing the study procedures in these two centres will make the results more generalizable beyond a few centres with specialized nutritional teams. Should this pilot study be successful, a larger trial will be planned with involvement of other PICUs within the Pediatric Acute & Critical Care Medicine Asian Network (PACCMAN).

Study participants

Children admitted to the PICUs BMI z-score $\leq$ -2 on PICU admission and who are anticipated to remain in the PICU long enough to benefit from protein supplementation will be considered for enrolment. Eligible children may be enrolled in this trial within 48 hours of starting feeds, provided feeding is started within the first 7 days of PICU admission. We chose to limit enrolment based on timing of feeds commencement because we hypothesize that early rather than delayed protein supplementation is important in modulating clinical outcomes. It is anticipated that children need to be exposed to the intervention for 5-7 days to accrue any potential benefit or to experience potential harms. The inclusion and exclusion criteria are summarized in Table 2. Children enrolled in a potentially confounding trial with biological interaction affecting outcome measures or adverse events will be excluded. However, if there are no identifiable biological interactions, the study team may consider co-enrolment in both trials.

Patient and Public Involvement

Patients and the public were not involved in the design of this protocol.

Risks, adverse events and consent

The potential for adverse events (AE) resulting from the proposed protein supplementation is expected to be minimal as the amount is within the current recommendations of major guidelines, albeit based on low-quality evidence. (15) Additionally, the design of this trial will seek to protect participants from harm by careful participant selection and appropriate monitoring. Through the exclusion criteria, we will be excluding children at highest risk for adverse effects. Extensive monitoring within the PICU will allow detection and treatment of any adverse effects that do occur including refeeding syndrome.

Monitoring and reporting of AEs and serious adverse events (SAEs) will be carried out in accordance with good clinical practice guidelines. Critically ill patients are at high risk of SAE and the usual approach of reporting all SAEs to the respective ethical boards would result in large numbers of reports of events not related to the trial intervention, but rather reflect the underlying disease process or expected complications of critical illness. (16) The most likely AEs associated with the study interventions are the development of feeding intolerance and diarrhoea, both of which are captured as outcomes and thus not reported as serious adverse events. Only SAEs that might reasonably be judged a consequence of participation in the trial and are judged by the investigators as not due to the underlying disease or expected complications of critical illness will be reported to the ethical boards. SAEs reporting will be performed within 24 hours to the reviewing ethical board and the data and safety monitoring board (DSMB).

Participants may be withdrawn from the study at any time due to an AE or SAE. These will be followed-up by the study team until the clinical outcome from the AE is determined. Examples include:

Prolonged feeding intolerance: Tolerating less than 50% of feeds prescribed over the period of ≥ 5 days
Development of acute kidney injury (according to KDIGO criteria) requiring dialysis(17)
Suspicion of enterocolitis
Significant gastrointestinal bleed requiring consideration for procedural intervention
On request by treating primary physicians

Research staff will approach the child's parents or legal guardians for consent of their child to participate in this trial (Appendix 1: Patient consent form). Potential benefits and risks will be written in the informed consent document. Patients and parents will be informed of the purpose, intervention, benefits and possible risks of the study. Whenever possible, assent will be obtained from children above 6 years old when the patient has emerged from a critical illness state.

Randomization, allocation concealment and blinding

Participants will be randomized to protein supplementation or standard care in a 1:1 ratio in undisclosed block size by sealed opaque envelope using a computer generated, centrally prepared allocation schedule by the study's biostatistics team. This randomization will be stratified according to centre. Clinical research coordinators or study team members will approach eligible patients for consent**.** Only after consented, will the study team members assign participants to allocated interventions – a model of prior consent will be adopted for this study.

All clinicians, bedside staff, and research staff involved in clinical management of the participants, parents and guardians will be unblinded to the treatment allocation.

Study interventions

This trial is an interventional study with two arms. Participants will be randomized to enteral protein supplementation or no enteral protein supplementation (i.e. standard nutrition care). For both trial arms, participants will be provided with enteral nutrition (EN) as per standard of care in each centre. General principles of the provision of EN using polymeric formula should include a stepwise progression of feeding volume individualized to the patient's weight, age and clinical status with close monitoring of tolerance by the nurses. Provision of EN values will be verified against nutritional requirements calculated by the dietitian. Children in both arms of the study will be fed so that the final feed volume will meet target energy requirements as calculated using the Schofield equation, with adjustments according to dietitian's assessment.(15) A 10% variation in energy intake per day will be allowed in both arms for ease of preparation of feeds.(18) Should feeding interruptions occur within either group due to clinical care, these will not be considered protocol violations.

Protein supplementation will be administered enterally and continue for a total of 7 days from study enrolment or until PICU discharge, whichever occurs earlier. Protein supplementation will consist of 100% whey protein isolate (Beneprotein ®, Nestle, Vevey, Switzerland). Protein supplementation will be provided in divided doses throughout the day and added to the prescribed milk formula feed regime to ensure a total protein intake of 1.5 g/kg/day when full feeds are achieved. For example, a child with a weight of 25kg receiving standard polymeric formula, would have an approximate intake of 40kcal/kg/day and 1.2g/kg/day protein. An additional 7.5g of protein is required, which is approximately equal to 1.25 scoops of protein supplement per day. Should a patient be prescribed with less than full feeds on a certain day (i.e. as feeds are graded up), protein supplementation will be proportionately administered.

If a recovering patient is able to take per oral solid feeds during the study intervention period, the intervention will be suspended due to the variability of oral dietary intake and difficulty in estimating protein and energy intake. If, however, a recovering patient no longer requires enteral feeding but continues to take per oral liquid/milk feeds, the intervention will continue until the stipulated timeframe. The study intervention will be stopped if the attending medical team believes withdrawal of the participant from the study is critical. At this stage, the treating team can follow their usual practice with respect to nutrition provision. Parents may also withdraw their child from the study at any point for any reason - should this occur, only data collected up to the point of withdrawal will be utilized in the analysis.

Data collection and management

Data collected will include baseline characteristics, PICU support therapies and detailed nutrition data (Table 1). The collection of nutrition data is a key component of this pilot study. Data pertaining to nutritional intakes of the participants will be collected. These include the following:

- Independent dietician estimation of energy (e.g., Schofield equation) and protein requirement
- EN volume delivered and corresponding calories and protein received
- Highest and lowest glucose levels in the first 24 hours and first week of PICU admission

- Daily fluid balance and electrolytes **(**if daily laboratory investigations are not clinically indicated, a minimum of 2 measurements will be done for the purposes of this study

Data will be extracted from electronic medical records by research staff who will enter the data directly into a secure web application (REDCap) hosted by Singapore Clinical Research Institute (SCRI).(19) The database will include both range checks and logic checks and will alert users to any missing data. The database will be stored at SCRI on a secure, firewall protected server with regular backups. Data can be entered by designated and trained users or survey respondents from any computer with an internet connection. User accounts incorporate electronic signatures comprised of a username and password. An audit trail is generated for all activity within each REDCap project.

Study outcomes

The pilot trial will focus on four primary feasibility outcomes and secondary clinical outcomes (Table 3). Change in muscle size and anthropometry will be measured in relation to measurements performed within 24hours of PICU admission as an exploratory outcome. Ultrasonography will be used to visualize and capture muscle changes in critically ill children (Appendix 2) (20). Change in functional status, as defined by the functional status scores (FSS) will be measured in relation to the pre-morbid function, and will be obtained from caregiver reports (Appendix 2) (21).

Sample size and interim analysis

The purpose of this pilot study is to investigate whether protein supplementation has promising efficacy and is worth further investigation. A large randomized study with usual care as the active control would be inappropriate as insufficient evidence of benefit of protein supplementation has yet to be obtained to justify such a study. In circumstances involving uncertainty of benefit and need for parsimony in resource expenditures, a small randomized study invoking the 'selection theory' approach proposed by Simon et al (22, 23) can provide an initial assessment of benefit. In the selection theory approach, the objective is to rank multiple potential treatments and then select those with the best responses for further study. However, our study involves only two treatments—protein supplementation versus standard feeds—which simplifies the approach in a determination of whether protein supplementation is better than standard feeds.

In the absence of any prior rates of clinical outcomes or effect size, our study will allow a response assessment and the potential for demonstrating greater efficacy of protein supplementation versus standard feeds in underweight critically ill children, with high statistical power, using a procedure that circumvents a formal hypothesis test.

Effect size is defined as $\delta = (\mu_1 - \mu_2)/\sigma$, where μ_1 and μ_2 represent clinical endpoint population means for the protein supplementation and standard feeds arms, respectively. In calculating sample size in the context of selection theory, we postulate the conventional underlying null and alternative hypotheses of H_0: $\delta \leq 0$ vs H_1: $\delta > 0$, respectively. In our pilot study, we will target an effect size of $\delta = 0.33$, which is considered a small-to-moderate effect size and often viewed as representing a clinically important difference. (24) If protein supplementation is superior to standard feeds by $\delta \geq 0.33$, we desire to detect this difference with power $\geq 90\%$. However, under a true null hypothesis, we will choose to ignore the type I error rate, and so set $\alpha = 50\%$—equivalent to random chance. Performing the sample size calculation based on a

one-sided hypothesis test of two independent means using a two-sample t-test with one-sided $\alpha = 0.50$, a sample size of $n = 35$ per group achieves power = 0.92 to detect an effect size of $\delta = 0.33$. (PASS® commercial software was used to perform the sample size calculation.)

From our preliminary data, we expect to have approximately 48 patients per year meet eligibility criteria for our pilot study. Our projection is that we will see 144 eligible patients over the 3-year recruitment period (3 x 48). Assuming a conservative consent rate of 55%, we anticipate at least 80 patients with BMI z-score ≤ -2 which will provide 40 patients in each study arm. Accounting for a dropout rate of 10-12% due to mortality and other causes would anticipate n = 35 patients per arm completing the study (total N = 70), which for $\delta \geq 0.33$ achieves > 90% probability for demonstrating protein supplementation superiority to standard feeds. To ensure we are able to assess feasibility and test study procedures and infrastructure at each site, we aim to enrol 26 or 27 patients per centre per year (13 or 14 per arm).

It is emphasized that under the selection theory paradigm, the best treatment for further consideration in a subsequent larger trial is selected on the basis of descriptive statistics—in this case, higher mean value. Hence, given an effect size of $\delta \geq 0.33$, the proposed procedure and sample size will ensure a > 90% probability of protein supplementation as the better treatment, demonstrated by a higher mean value, without a formal hypothesis test. A 95% confidence interval will be calculated on the protein supplementation versus standard feeds mean difference for the clinical efficacy variables.

Should recruitment be slow and challenging, the study team will meet and decide on the best method in increase enrolment. Some *a priori* strategies that we will consider include (but not limited to) changing the criteria to include:

- Children on non-invasive ventilation or respiratory distress, and requiring any form tube-feeding
- Children with BMI ≤ -1 on PICU admission

Statistical analysis

All analyses will be performed using an intention-to-treat principle. There will be no interim efficacy analyses for this pilot trial. If, after the completion of the pilot trial, the study team determines that there are no important changes to the inclusion and exclusion criteria, the results will not be unblinded for the clinical outcomes of the pilot trial (Figure 1). Instead, we will report the feasibility outcomes, present the clinical outcomes as a single cohort, and consider the pilot trial to be an internal pilot, meaning that we will include the pilot trial patients in the larger RCT. If the study team determines a large trial is not feasible or if including the pilot trial patients in the larger RCT is inappropriate, the clinical outcomes and group comparisons will be reported so that the trial can be included in future meta-analyses. We will use the CONSORT guidelines for reporting.(25, 26)

Feasibility Analysis

Feasibility will be demonstrated by (1) achieving recruitment targets (effective screening, timely enrolment and satisfactory participant accrual), (2) demonstrating at least 80% regimen compliance to allocated groups, (3) demonstrating safety of the intervention and (4) demonstrating delivery of protein with a separation of at least a 0.5g/kg/day in the intervention and control arms. Effective screening will be achieved if 90% of all PICU admissions are screened within 24hours, timely enrolment will be achieved if 90% of all eligible participants are enrolled within 48hours of meeting eligibility criteria and satisfactory participant accrual is considered if both centres recruit a total of at least 26 patients per year.

For the feasibility outcomes we will report the proportions of children meeting each criterion and the associated 95% confidence intervals. We will also compare total protein received by participants in the groups. We chose a separation of 0.5g/kg/day protein as clinically meaningful based on our data from two cohorts of critically ill patients [bronchiolitis (27) and acute respiratory distress syndrome (9)] which demonstrated that without supplementation, the median protein achieved within the first 3 days of illness was < 1.0g/kg/day and that 0.5g/kg/day separation was associated with improved clinical outcomes, respectively. The number of participants who consented (or not consented) and completed (or discontinued early) the study and the reasons for non-consent/ discontinuation will be summarized using counts and percentages. Demographic and baseline characteristics will also be summarized using descriptive statistics. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of protein supplementation.

Clinical Outcome Analysis

PICU and hospital mortality rate in each arm and differences between the protein supplementation and standard of care arms will be presented with exact 95% confidence intervals. Medians of continuous variables [PICU LOS, hospital LOS, MV duration, 28-day ventilator-free days (VFD) and PICU-free days (IFD)] will be presented along with corresponding 95% confidence intervals. LOS and duration endpoints will be compared between treatment groups using a log-rank test in conjunction with Kaplan-Meier survival curves. Patients who die will be censored. If warranted, additional analysis using Cox regression will be performed to adjust for the influence of potential demographic and clinical confounders

Differences in total hospital LOS, PICU stay, duration of MV, VFD and IFD observed in the protein supplementation group relative to the standard care group will be assessed by subgroup according to illness severity level as characterized by PIM3 scores (28, 29). Change in muscle size (e.g., ultrasound guided cross-sectional area of the rectus femoris), anthropometry (height, weight, BMI) and functional status (PEDI-CAT score, FSS score, hand-grip strength and 6-minute walk test) during PICU stay, at PICU discharge, hospital discharge and 6-months later will also be measured as exploratory outcomes.

Handling of missing data

Baseline characteristics, PICU support therapies, nutrition and outcome data will be recorded in the electronic medical record system. Therefore, data is very unlikely to be missing. Trained clinical research coordinators will enter data into the REDcap system which will have both range checks and logic checks and alert users to any missing data. If data are still missing, no imputation will be done.

Trial steering committee

There will be a single steering committee overseeing trial execution over the two participating sites. The committee will consist of the two site-principal investigators, two dietitians, two nursing leads and four study team members representative from both sites. This group will be responsible for each step of the trial process including ensuring consistent screening, reviewing recruitment numbers, deliberating on eligibility of participants and adverse events. The steering committee will meet quarterly to discuss progress of the trial and troubleshoot any problems or delays in the project plan.

Data safety monitoring

An independent Data Safety Monitoring Committee (DSMC) comprised of three members with experience and expertise in methods, statistics and critical care collectively will monitor the progress and safety of the trial. The DSMC will meet and review the available data when 30% of randomized patients (total of 20 patients or at least 10 in each arm) have completed one month of follow-up. Additional meetings may be held at the discretion of the Chair of the DSMC. The committee will receive SAE reports as they occur. All data will be presented to the DSMC tabulated by intervention group, but the members will remain blinded to the actual group assignment. The committee will review SAEs and centre performance (enrolment, data quality and protocol adherence) and any pertinent external data such as newly published studies or other potentially relevant safety information. They may recommend early termination of the trial if there are SAEs associated with the trial intervention, but no formal stopping rules will be used: this decision will be based on clinical judgment of the DSMC. The DSMC will keep all trial data, committee deliberations and meeting minutes confidential until the end of the trial.

Discussion

Though primarily designed to assess feasibility, this study will be the first RCT investigating the benefits/risks of protein supplementation in addition to standard nutrition in critically ill children. Continuation of this pilot trial into the definitive multicentre RCT will address an important scientific hypothesis—does early enteral protein supplementation of 1.5g/kg/day improve clinical, functional and nutritional outcomes in critically ill children. Numerous prior observational studies with similar aims (5, 9) were inadequately controlled for important selection biases, that is, sicker patients selectively received less nutrition (including less protein). As such, drawing a conclusion that higher nutrition (including higher protein) intake is associated with improved outcomes is inherently biased. A randomized design, such as the proposed study, is the only way to control for such bias.

In critical illness (e.g., sepsis, major surgery), changes in endocrine-metabolic responses lead to an imbalance in protein synthesis and degradation.(30) A negative protein balance is associated with immunosuppression, poor wound healing, loss of lean muscle mass and a delay in the recovery process.(31) Muscle catabolism is inevitable in acute illness and its intensity depends on the severity of illness. (30) With exogenous nutritional protein and sufficient energy intake, it is postulated that lean muscle mass can be diverted away from oxidative metabolic pathways and preserved. (32) It is, however, unknown what constitutes the optimal amount of protein required to minimize loss of lean muscle mass and the optimal timing of administration in relation to critical illness. Prevailing data from adult studies demonstrate benefits (improved muscle mass (33), reduced mortality (34, 35), as well as, harm (muscle wasting (12), increased mortality (36) associated with protein intake in critical illness. These adult data cannot be extrapolated to children, whose protein and energy requirements are inherently different. (37)

There are currently several recommendations for protein requirements during critical illness. The 2018 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommends 1.3g/kg/day protein equivalents be delivered in critically ill adults.(38) In contrast, the American Society for Parenteral and Enteral Nutrition (ASPEN) in conjunction with the Society of Critical Care Medicine (SCCM) 2016 guidelines for critically ill adults recommends 1.2-2.0g/kg/day of protein intake.(39) In critically ill children the recommended

protein requirement according to the ASPEN 2017 guidelines was 1.5g/kg/day, acknowledging that the optimal protein intake required to attain a positive protein balance may be much higher than this minimum threshold.(40) It was also suggested that provision of protein early in the course of critical illness was desirable to promote positive nitrogen balance. (40) The provision of 1.5g/kg/day of enteral protein in our intervention arm is based on these guidelines and on translational studies indicating that at least 1.5g/kg/day of protein was required to equilibrate nitrogen and energy balances in critically ill children. (41, 42) It is noteworthy, however, that the PEPaNIC trial (early vs. late parenteral nutrition in critically ill children) comparing nutrition supplementation in the form of early parenteral nutrition within 24 hours of PICU admission vs. late supplementation with parenteral nutrition after the first week of PICU stay demonstrated a higher rate of new infection, prolonged PICU stay and decreased likelihood of being discharged alive from hospital in the early group. In the PEPaNIC trial, the early group received higher protein intake (approximately 1.5g/kg/day) in the form of an intravenous amino acid solution over the first week of PICU stay (43). There are, however, fundamental differences between the current proposed study and PEPaNIC trial which make direct extrapolation of outcomes inappropriate. Firstly, the PEPaNIC trial included critically ill children "at-risk of malnutrition" [using the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids)], whereas, we chose to focus on established underweight patients (BMW z-score $\leq$ -2) who have the greatest potential to benefit from nutritional therapy. (44) Secondly, the PEPaNIC trial utilized parenteral nutrition instead of EN which in itself has been associated with infections and other poor outcomes. (45-47) As such, an empirical trial of supplemental enteral protein is warranted and will be informative.

Despite the benefits of a randomized design, our pilot RCT may be susceptible to some potential bias. In this dual center RCT, there is no standardized EN protocol between the two centers. We did not mandate application of a standardized enteral nutrition protocol across both sides because we aim to scale up and conduct a larger pragmatic trial if feasibility is demonstrated. It will be challenging to perform a larger trial with a standardized enteral nutrition protocol across multiple sites. Though routine protein supplementation is not currently practiced in both centers, the variable practice may lead to potential overlap in protein dosing between the intervention and control arms. We recognize this as a limitation but are unable to justify ethically to reduce protein intake of patients to below what standard care provides. In addition, a proportion of patients will be excluded from the study due to safety concerns (exclusion criteria) and this will limit the generalizability of this RCT. The pragmatic design of this study also allows the managing clinical team (including nurses and physicians) and investigators in charge of enrolling participants to be unblinded to the intervention. However, blinding will be maintained for all other research staff, such as statisticians. As indirect calorimetry is not readily available at both sites, energy equations would be used to calculate requirements, which could result in energy over or underfeeding. (48) Non-protein calories which may in itself indirectly affect protein catabolism (49) and clinical outcomes (35, 50), will be recorded and analysed but will not be strictly controlled. Lastly, sedation practices, physical activity (51, 52) and early rehabilitation (53) (which are challenging to control) may interact with nutritional therapy to affect clinical, nutritional and functional outcomes measured in this study.

Trial status

This trial has obtained ethics approval and clinical trial registration. Patient recruitment is anticipated to begin on 4th January 2021 and to complete on 3rd January 2024. Follow-up will be completed by 30th June 2024.

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Contributors
JHL, JJMW, JSMO, CSO, JCA and FKC conceived and designed this study. JHL, JCA and MG designed the statistical plan. JHL, JJMW, JSMO and CSO obtained permission from the ethics committees. JHL, JJMW, JSMO, CSO, JCA, FKC, LJF, RT, JKBL and PFP will carry out this trial. JHL, JJMW, CSO and JCA drafted this manuscript. JSMO, FKC, LJF, RT, JKBL and PFP carefully reviewed the manuscript; and all authors read and approved the final manuscript.

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Ethics approval
This study has been reviewed and approved by the SingHealth Centralised Institutional Review Board (CIRB) and National Healthcare Group Domain Specific Review Board (DSRB). The study will be conducted in adherence to Singapore Good Clinical Practice (GCP) guidelines. This trial does not need approval with the Health Science Authority of Singapore. This study is registered with clinicaltrials.gov (NCT04565613).

Competing interest
Nil

Patient consent for publication
Not required

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Table 1: SPIRIT schedule of enrolment, interventions, and assessments

Study phase		Screening phase		Treatment phase								Follow-up phase		
Scheduled timeline (from the day of randomization)		**D -7 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**EOT***	**PICU discharge**	**Hospital discharge**	**6 months**
Inclusion/exclusion criteria	**PICU admission**	•												
Demographics[a]		•												
Medical/surgical history		•												
Informed consent		•												
Randomization			•											
Allocation			•											
Calculation of protein supplementation			•											
Investigational product administration														
Protein Supplementation + Standard nutrition*				•	•	•	•	•	•	•				
Standard nutrition (control arm)				•	•	•	•	•	•	•				
Data collection														
Feasibility data[b]		•	•	•										
Clinical data[c]		•	•	•	•	•	•	•	•	•		•	•	
Nutrition data[d]				•	•	•	•	•	•	•				
Laboratory assessment														
Blood sample: blood sugar[e]				•			•**							
Blood sample: renal panel[f]				•			•**							
Outcome assessments														
Clinical outcomes[g]												•	•	•
Safety assessments														
Physical examination[h]				•	•	•	•	•	•	•				
Vital signs[i]				•	•	•	•	•	•	•				
Adverse and serious events collection[j]				•	•	•	•	•	•	•	•	•	•	•
Muscle mass and functional status														
Muscle US[k]					•					•		•	•	•
Functional Status Scale Score					•					•		•	•	•
PEDI-CAT					•					•		•	•	•

EOT: end of treatment
PICU: Pediatric Intensive Care Unit
US: Ultrasound
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test.

*Patients will be considered to have reached EOT based on the following:
Complete 7 days of protein supplementation, PICU discharge, the patient has recovered enough to start oral solid feeds, the attending medical team withdraws the patient from the study, death

**Results of blood glucose and renal panel throughout the week, done for clinical indications, will be recorded. If none are clinically indicated, a minimum of 2 measurements will be done for the purposes of this study

[a]Demographics: Age, weight, height, midarm circumference
[b]Feasibility data: proportion of eligible patients approached for consented, number of patients receiving intervention by 72 hours of enrolment, adherence to intervention protocol
[c]Clinical data: baseline characteristics, severity score (Pediatric Index of Mortality 3), PICU support therapies
[d]Nutrition data: nutritional requirements will be calculated (Schofield for calories and 1.5g/kg/day for protein), nutrition prescribed and delivered (calories, protein, carbohydrate, fat, micronutrients) for enteral and parenteral nutrition, fluid input and output
[e]Blood sugar: measurement from bedside finger-prick glucose meter or plasma glucose, on at least three occasions
[f]Renal panel: serum urea, sodium, potassium, chloride, bicarbonate and creatinine
[g]Clinical outcomes: PICU mortality, PICU length of stay, hospital length of stay, duration of ventilation
[h]Physical examination: evaluation of the cardiovascular, respiratory, abdominal, genitourinary, neurological and musculoskeletal system
[i]Vital signs: heart rate, systolic and diastolic blood pressure, body temperature, respiratory rate, oxygen saturation and pain score
[j]Adverse and serious adverse events includes but not limited to prolonged feeding intolerance (tolerating <50% feeds for ≥ 5days, development of acute kidney injury requiring dialysis, suspicion of enterocolitis, gastrointestinal hemorrhage requiring procedural intervention. If the adverse/serious adverse event is related to the investigational product, participants may be withdrawn and followed up by the study team until clinical outcome of the adverse event is determined
[k]Muscle ultrasound: baseline measurement of rectus femoris cross-sectional area and diaphragm thickness will be taken within 72 hours of enrolment

Table 2: Inclusion and exclusion criteria

Inclusion criteria:	Children (28 days to 18 years of age) Both elective or emergency admissions BMI z-score ≤ -2 on PICU admission Invasive MV beginning within 48 hours of PICU admission and anticipated to continue for ≥ 48 hours Enteral nutrition support for feeding (e.g., orogastric, nasogastric, gastrostomy, nasojejunal, orojejunal)
Exclusion criteria:	Contraindications to enteral nutrition (e.g., gut hemorrhage, post-gastrointestinal surgery, necrotizing enterocolitis, ischemic bowel etc.) Cow's milk protein allergy* Anorexia nervosa and other eating disorders Premature infants (corrected gestational age of < 44 weeks) Parenteral nutrition Extra-corporeal membrane oxygenation Conditions requiring significant fluid restriction (≤75% of maintenance fluids) (e.g., post cardiac surgery) Progressive neuromuscular disease (e.g., spinal muscular atrophy, Duchenne or other muscular dystrophy, multiple sclerosis, amyotrophic lateral sclerosis) Medical conditions where increased or decreased protein intake is required, including acute kidney injury (stage 3 KDIGO criteria), chronic kidney disease (stage 4 and 5), inborn errors of metabolism, fulminant liver failure, severe burn injury Patients who are not expected to survive this PICU admission (e.g., palliative care, do-not-resuscitate orders, limitation of care orders) Previously enrolled in this trial Enrolled in a potentially confounding trial

* The protein supplement used in our study, as well as, most standard polymeric formulas are contraindicated in patients with cow's milk protein allergy
BMI: Body mass index
PICU: Pediatric Intensive Care Unit
MV: Mechanical ventilation
KDIGO: Kidney Disease Improving Global Outcomes

Table 3: Study outcomes

Primary feasibility outcomes	Proportion of eligible patients approached for consent Proportion of participants receiving their first protein supplementation within 72 hours of enrolment Participant accrual, defined as an average monthly enrolment of at least one participant per centre Protocol adherence, defined as >80% of protein target administered according to the protocol in the intervention arm
Secondary clinical outcomes	PICU mortality PICU LOS 28-day PICU-free days Hospital LOS MV duration 28-day ventilator-free days Development of AEs including feeding intolerance, diarrhoea, GI bleeding, and treatment used for GI bleeding Change in muscle size (e.g., ultrasound guided cross-sectional area of the rectus femoris, diaphragm thickness) during PICU stay, at PICU discharge, hospital discharge and 6-months later Change in anthropometric measurements (height, weight, BMI) at PICU discharge, hospital discharge and 6-months later Change in functional status (PEDI-CAT score, FSS score, hand-grip strength and 6-minute walk test) at hospital discharge and 6-months later

PICU: Pediatric Intensive Care Unit
LOS: Length of Stay
MV: Mechanical ventilation
AE: Adverse Effects
GI: Gastrointestinal
BMI: Body mass index
PEDI-CAT: Pediatric Evaluation of Disability Inventory – Computer Adaptive Test
FSS: Functional Status Score

Figure legends

Figure 1: Flowchart for analytical approach of pilot trial

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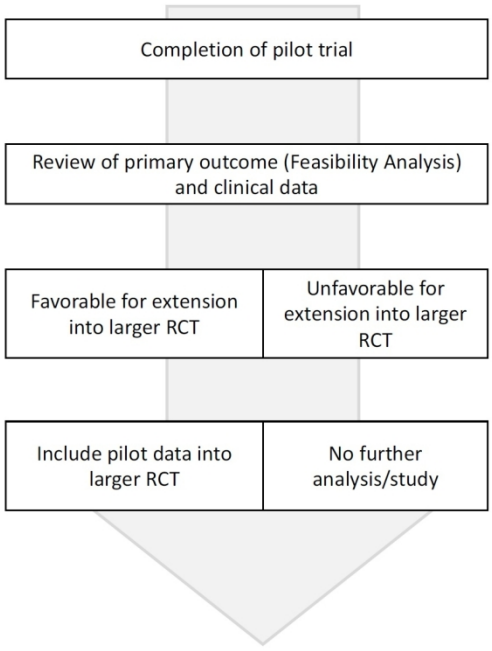

Figure 1: Flowchart for analytical approach of pilot trial

468x345mm (96 x 96 DPI)

APPENDIX 1: PATIENT CONSENT FORM

SingHealth

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

You are being invited to participate in a research study. Your child's participation in this study is entirely voluntary. Before you agree for your child to take part in this research study, the study must be explained to you and you must be given the chance to ask questions. Your questions will be answered clearly and to your satisfaction. Please read carefully the information provided here. If you agree to participate, please sign the consent form. You will be given a copy of this document.

STUDY INFORMATION

Protocol Title:
Protein Supplementation versus Standard Feeds in Critically Ill Children: A Dual-Centre Randomized Controlled Pilot Trial

Principal Investigator:
Dr. Lee Jan Hau
Children's Intensive Care Unit
KK Women's and Children's Hospital

PURPOSE OF THE RESEARCH STUDY

A large study in many hospitals is needed to test whether not giving additional protein to sick children improves outcomes. Large studies in children are very hard to do but very important. This current study is a pilot trial. This means that it is a smaller study to test whether it is possible to do a larger study. We hope to learn how best to do a larger study. If your child takes part in this study, their data may be included in a larger study in the future.

Your child was selected as a possible participant in this study because he or she is in the Children's Intensive Care Unit (CICU), needs a breathing tube and assistance in feeding. All critically ill children receive nutrition when are in the CICU. However, the best nutrition plan is still not known. We aim to study whether giving more proteins in the feeding will help improve outcomes in these children.

This study targets to recruit 45 participants from KK Women's and Children's Hospital. About 70 participants are expected to take part in this study at two hospitals in Singapore.

STUDY PROCEDURES & YOUR RESPONSIBILITIES IN THIS STUDY

If you agree for your child to take part in this study, your child will be given feeding with or without additional protein for up to 7 days. Your child's participation in the study will last up to 6 months from the time of discharge from the hospital.

Your child will be given the allocated nutrition for about 7 days, have some tests [for example, muscle ultrasound and strength assessment (Table 1)] performed during his or her stay in the CICU and be followed up for 6 months. After discharge, your child will need to visit the doctor's office once in the course of the study.

In addition, some health information will be collected from your child's medical records. The information include the basic demographic data, the clinical data as part of routine clinical monitoring of any critically ill child on enteral nutrition, intensive care support data, clinical outcome and etc.

Table 1: Study Assessments

Assessments	Baseline	CICU discharge	Hospital discharge	6 months
Body measurements - Examples: Height, weight, mid arm circumference	✓		✓	✓
Muscle ultrasound - A scan to measure muscle size	✓	✓	✓	✓
Assessments of daily activities - A series of questions to measure abilities in daily activities, mobility and social abilities	✓	✓	✓	✓
Hand-grip strength test (if > 6 years old) - A simple test to measure general strength by asking your child to squeeze the measuring tool as hard as possible		✓	✓	✓
6-minute walk test (if > 6years old) - A simple test to measure the maximum distance your child can walk in 6 minutes			✓	✓

If you agree for your child to take part in this study, your child will be randomised to receive standard milk feeds or milk feeds with additional protein. Randomisation means assigning your child to one of two groups by chance, like tossing a coin or rolling dice. The study team, your child's doctors, nurses and yourself will know which group your child is in.

If you agree for your child to participate in this study, you should follow the advice and directions given to you by the study team.

WHAT IS NOT STANDARD CARE OR IS EXPERIMENTAL IN THIS STUDY

The study is being conducted because addition of protein is not yet proven to be a standard treatment in sick children in the CICU. We hope that your child's participation will help us to determine whether additional protein is equal or superior to existing feeding practice.

The study will involve the use of randomisation (assignment of which group by chance), which is usually only done for research studies.

Although addition of protein may be part of standard medical care in certain situations, in this study, the addition of protein (if your child is assigned to the protein group) and the follow up tests and assessments (Table 1) are being performed for the purposes of the research and are not part of your child's routine care.

POSSIBLE RISKS, DISCOMFORTS OR INCONVENIENCES

Muscle Ultrasound
Ultrasound scan is safe and non-invasive. However, your child may possibly feel a slight discomfort during the scan from the contact of the ultrasound probe and the gel to the skin surface.

Hand-grip strength test
Your child may possibly feel uncomfortable as he/she has to squeeze the measuring tool as hard as possible.

6-minute walk test
Your child may possibly feel breathlessness or giddiness during the walk.

Assessments of daily activities
Some of the questions might make you/your child feel uncomfortable or upset. You/your child may refuse to answer any of the questions and/or take a break at any time during the study.

Personal privacy and confidentiality:
This study uses health information that may affect your child's privacy. To protect your child's confidentiality, only a unique code number will be used to identify data that we collected from your child.

As there will be a link between the code and your child's identifiable information, there is still a possibility of data breach. A data breach is when someone sees or uses data without permission. If there is a data breach, someone could see or use the data we have about your child. Even without your child's name, there is a chance someone could figure out who is your child. They could misuse your child's data. We believe the chance of this is very small, but it is not zero.

POTENTIAL BENEFITS

There is no assurance that your child will benefit from this study. However, your child's participation may add to the medical knowledge about the use of additional protein in the providing for good nutrition care in sick children in the CICU.

ALTERNATIVE PROCEDURES/ TREATMENTS IF YOU DO NOT PARTICIPATE IN THE STUDY

If you choose not to take part your child in this study, the alternative is to have what is considered standard care for your child's condition. In our institution, this would be feeding ordered and provided by the medical and nursing team. You may discuss the possible risks and benefits of the alternatives with your child's doctor.

COSTS & PAYMENTS IF PARTICIPATING IN THIS STUDY

There is no cost to you for your child participating in this research study.

If you agree for your child to take part in this study, the following will be performed at no charge to you:

1. Muscle ultrasound
2. Assessment of function and physical strength at follow-up visit (i.e. assessment of daily activities, hand-grip strength test, 6-minute walk test)

These costs will be borne by KK Women's and Children's Hospital

The cost of your child's usual medical care (procedures, medications and doctor visits) will continue to be billed to you.

You will be reimbursed for your time, inconvenience and transportation costs as follows:

- If you complete the study, you will receive SGD 50

INCIDENTAL FINDINGS

During the course of the study, there is a possibility that we might unintentionally come to know of new information about your child's health condition from ultrasound that is being performed as part of the study. These are called "incidental findings".

"Incidental findings" are findings that have potential health or reproductive importance to a participant like your child and are discovered in the course of conducting the study, but are unrelated to the purposes, objectives or variables of the study. These findings may cause you and your child to feel anxious and may affect your child's current or future life and/or health insurance coverage. Examples of potential incidental findings that may be discovered during the course of this study may include but are not limited to muscle abnormalities or growths. You will be asked to indicate whether you wish to be re-identified and notified in the event of an important incidental finding that is related to you.

If you agree to be re-identified and notified, your study doctor/ a qualified healthcare professional will explain the incidental finding to you and discuss and advise you on the next steps to follow. You may wish to do more tests and seek advice to confirm this incidental finding. The costs for any care that will be needed to diagnose or treat an incidental finding would not be paid for by this research study. These costs would be your responsibility.

If you do not wish to be re-identified and notified, your decision will be respected. However, in exceptional situations such as discovery of life-threatening incidental findings with available treatment options, you will be contacted to confirm your decision whether to learn more about the incidental findings. In rare situations where the incidental findings have public health implications and as required by the law (e.g. under the Infectious Diseases Act), you will be contacted and informed of the incidental findings.

PARTICIPANT'S RIGHTS

Your child's participation in this study is entirely voluntary. You have a right to ask questions, which the study team will do their best to answer clearly and to your satisfaction.
In the event of any new information becoming available that may be relevant to your willingness to continue your child in this study, you (or your legal representative, if relevant) will be informed in a timely manner by the Principal Investigator or his/her representative and will be contacted for further consent if required.

WITHDRAWAL FROM STUDY

You are free to withdraw your consent and discontinue your child's participation in the study at any time, without your child's medical care being affected. If you decide to stop your child taking part in this study, you should tell the Principal Investigator.

If you withdraw from the study,

- Your child will continue to receive standard medical care as per the primary team
- Feeding plan will continue as per standard medical plan by the primary team

However, any of your child's data that has been collected until the time of your withdrawal will be kept and analysed. The reason is to enable a complete and comprehensive evaluation of the study.

Your child's study doctor, the Principal Investigator of this study may stop your child's participation in the study at any time for one or more of the following reasons:

- Failure to follow the instructions of the Principal Investigator and/or study staff.
- The Principal Investigator decides that continuing your child's participation could be harmful to your health or safety.
- Pregnancy
- Your child requires treatment not allowed in the study.
- The study is cancelled.

RESEARCH RELATED INJURY AND COMPENSATION

If you follow the directions of the Principal Investigator of this research study and your child is injured due to the research procedure given under the plan for the research study, our institution will provide you with the appropriate medical treatment.

Payment for management of the normally expected consequences of your child's treatment (i.e. consequences of your treatment which are not caused by your child's participation in the research study) will not be provided.

You still have all your legal rights. Nothing said here about treatment or compensation in any way alters your right to recover damages where you can prove negligence.

CONFIDENTIALITY OF STUDY AND MEDICAL RECORDS

Your child's participation in this study will involve the collection of Personal Data. "Personal Data" means data about your child which makes him/her identifiable (i) from such data or (ii) from that data and other information which an organisation has or likely to have access. Examples of personal data include name, national registration identity card (NRIC), nationality, passport information, date of birth, and telephone number.

Personal Data collected for this study will be kept confidential. Your child's study records and medical records, to the extent required by the applicable laws and regulations, will not be made publicly available. Only the study team will have access to the personal data being collected from your child. In the event of any publication regarding this study, your child's identity will remain confidential.

However, the monitor(s), the auditor(s), the Institutional Review Board, and the regulatory authority(ies) will be granted direct access to your child's original medical records and study records to verify study procedures and data, without making any of your information public.

By signing the Consent Form, you consent to (i) the collection, access to, use and storage of your child's Personal Data by KK Women's and Children's Hospital, and (ii) the disclosure of such Personal Data to our authorised service providers and relevant third parties as mentioned above.

Any information containing your child's Personal Data that is collected for the purposes of this research will be stored in Singapore. To protect your child's identity, his/her Personal Data will be labelled with a unique code number. The code will be used in place of your child's name and other information that directly and easily identifies him/her. The study team will keep a separate file that links your child's code number to his/her Personal Data. This will be kept in a safe place with restricted access.

All data collected in this study are the property of KK Women's and Children's Hospital. The data will be used for the purpose of this pilot study and for the future larger study, if the study teams find that it is feasible to conduct the larger study. For this purpose, consent for future research will be sought from you.

By participating in this research study, you are confirming that you have read, understood and consent to the SingHealth Data Protection Policy, the full version of which is available at www.singhealth.com.sg/pdpa.

WHO HAS REVIEWED THE STUDY

This study has been reviewed by the SingHealth Centralised Institutional Review Board for ethics approval.
If you have questions about your rights as a participant, you can call the SingHealth Centralised Institutional Review Board at 6323 7515 during office hours (8:30 am to 5:30pm).

WHO TO CONTACT IF YOU HAVE QUESTIONS REGARDING THE STUDY

If you have questions about this research study or in the case of any injuries during the course of this study, you may contact:

Principal Investigator
Dr. Lee Jan Hau
Children's Intensive Care Unit, KK Women's and Children's Hospital
+65-63941778
+65-62255554

If you have any feedback about this research study, you may contact the Principal Investigator or the SingHealth Centralised Institutional Review Board.

CONSENT FORM FOR RESEARCH STUDY

Protocol Title:
Protein Supplementation versus Standard Feeds in Critically Ill Children: A Dual-Centre Randomized Controlled Pilot Trial

Principal Investigator:
Dr. Lee Jan Hau
Children's Intensive Care Unit, KK Women's and Children's Hospital

To be completed by participant
(For child who is 13 years old and above, and of normal mental capacity, and when he/she is in stable condition)

I agree to participate in the research study as described and on the terms set out in the Participant Information Sheet.

The nature, risks and benefits of the study have been explained clearly to me and I fully understand them.

I understand the purpose and procedures of this study. I have been given the Participant Information Sheet and the opportunity to discuss and ask questions about this study and am satisfied with the information provided to me.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reasons and without my medical care being affected.

By participating in this research study, I confirm that I have read, understood and consent to the SingHealth Data Protection Policy.

_____	_____	_____
Name of participant	Signature/Thumbprint (Right / Left)	Date of signing

To be completed by parent / legal guardian / legal representative

I agree for _____ (Name of Participant) to participate in the research study as described and on the terms set out in the Participant Information Sheet.

The nature, risks and benefits of the study have been explained clearly to me and I fully understand them.

I understand the purpose and procedures of this study. I have been given the Participant Information Sheet and the opportunity to discuss and ask questions about this study and am satisfied with the information provided to me.

I understand that my child's participation is voluntary and that I am free to withdraw my child at any time, without giving any reasons and without my child's medical care being affected.

By participating in this research study, I confirm that I have read, understood and consent to the SingHealth Data Protection Policy.

Consent to be Re-identified and Notified in the case of an Incidental Finding

There may be potential incidental findings arising from this research. Please indicate whether you consent to re-identification and notification about the incidental finding:

☐ Yes, I wish to be re-identified and notified in the case of an incidental finding from this research. I can be reached by:

Phone/ Email:

☐ In the event that I cannot be reached, please contact the following person nominated by me: [Optional]

Name/ Phone/ Email:

☐ No, I do not wish to be re-identified and notified in the case of an incidental finding from this research. However, I understand that in exceptional or rare situations, I will be contacted as described in the Participant Information Sheet:

- In exceptional situations such as discovery of life-threatening incidental findings with available treatment options, I will be contacted to confirm my decision whether to learn more about the incidental findings.
- In rare situations where the incidental findings have public health implications and as required by the law (e.g. under the Infectious Diseases Act), I will be contacted and informed of the incidental findings.

Name of participant's parent/ legal guardian/ legal representative	Signature/Thumbprint (Right / Left)	Date of signing

To be completed by translator, if required

The study has been explained to the participant/ legal representative in

_____ by _____.
Language Name of translator

To be completed by witness, where applicable

I, the undersigned, certify that:

- I am 21 years of age or older.
- To the best of my knowledge, the participant or the participant's legal representative signing this informed consent form had the study fully explained to him/her in a language understood by him/ her and clearly understands the nature, risks and benefits of the participant's participation in the study.
- I have taken reasonable steps to ascertain the identity of the participant or the participant's legal representative giving the consent.
- I have taken reasonable steps to ascertain that the consent has been given voluntarily without any coercion or intimidation.

Witnessed by: _____ _____
Name of witness Date of signing

_____
Signature of witness

1. An impartial witness (who is 21 years of age or older, has mental capacity, who is independent of the research study, and cannot be unfairly influenced by people involved with the research study) should be present during the entire informed consent discussion if a participant or the participant's legal representative is unable to read, and/or sign and date on the consent form (i.e. using the participant's or legal representative's thumbprint). After the written consent form and any written information to be provided to participant is read and explained to the participant or the participant's legal representative, and after the participant or the participant's legal representative has orally consented to the participant's participation in the study and, if capable of doing so, has signed and personally dated the consent form, the witness should sign and personally date the consent form. This is applicable for Clinical Trials regulated by HSA and Human Biomedical Research under the HBRA.

2. For HBRA studies, the witness may be a member of the team carrying out the research only if a participant or the participant's legal representative is able to read, sign and date on the consent form.

Investigator's Statement

I, the undersigned, certify to the best of my knowledge that the participant/ participant's legal representative signing this consent form had the study fully explained to him/her and clearly understands the nature, risks and benefits of the participant's participation in the study.

Name of Investigator/ Person obtaining consent	Signature	Date

INFORMATION & CONSENT FORM FOR FUTURE RESEARCH

This is an optional component that is separate from the research study. Your child may still participate in the research study if you say "No" to this. Please ask questions if you do not understand why we are asking for your permission.

In this Consent Form for Future Research, we seek your permission to keep your child's data for future research. The data will be kept in KK Women's and Children's Hospital. Except if you withdraw your consent or there are limits imposed by law, there is no limit on the length of time we will store your data. Researchers will use your child's data for research long into the future.

This is what will be done with your child's stored data:

- We may use the data to answer additional research questions in other research studies. This is outside the scope of the research study but still related to nutrition in critically ill children.
- We may share the data with other researchers at National University Hospital, Singapore and with researchers outside of Singapore (Pediatric Acute & Critical Care Medicine Asian Network.)
- The stored data will be labelled with a code instead of information that directly identifies your child (e.g. name, NRIC, date of birth, etc.). We will keep a separate file (key) that links your child's code to his/her identifiable information.
- When we share your child's data with other researchers, it will be in a coded manner. They will not be able to identify your child from the coded data.
- If you decide at a later time that you do not want your child's data to be used for future research, you can contact the Principal Investigator or study team at any time. All your child's stored data that has not been used or shared with other researchers will be removed and discontinued from further use, unless this information is already included in analyses or used in publications.

CONSENT FORM FOR FUTURE RESEARCH

To be completed by participant
(For child who is 13 years old and above, and of normal mental capacity, and when he/she is in stable condition)

This component is optional. You do not have to agree to it in order to participate in the research study.

Please indicate your choice using the relevant checkbox.

☐ I agree to have my data stored for future use in other research studies.
☐ I do not agree to have my data stored for future use in other research studies.

I understand the purpose and nature of this optional component (storage of data for future use in other research studies). I have been given the Information & Consent Form for Future Research and the opportunity to discuss and ask questions about this optional component and am satisfied with the information provided to me.

I confirm that I have read, understood and consent to the SingHealth Data Protection Policy.

Name of participant	Signature/Thumbprint (Right / Left)	Date of signing

To be completed by parent / legal guardian / legal representative

This component is optional. You do not have to agree to it in order for _____ (Name of Participant) to participate in the research study.

Please indicate your choice using the relevant checkbox.

☐ I agree to have my child's data stored for future use in other research studies.
☐ I do not agree to have my child's data stored for future use in other research studies.

I understand the purpose and nature of this optional component (storage of data for future use in other research studies). I have been given the Information & Consent Form for Future Research and the opportunity to discuss and ask questions about this optional component and am satisfied with the information provided to me.

I confirm that I have read, understood and consent to the SingHealth Data Protection Policy.

Name of participant's parent/ legal guardian/ legal representative	Signature/Thumbprint (Right / Left)	Date of signing

To be completed by translator, if required

The optional component (storage of data for future use in other research studies)has been explained to the participant/ participant's legal representative in

_____ by _____.
Language Name of translator

To be completed by witness, where applicable

I, the undersigned, certify that:

- I am 21 years of age or older.
- To the best of my knowledge, the participant or the participant's legal representative signing this Information & Consent Form for Future Research had the optional component fully explained to him/her in a language understood by him/ her and clearly understands the purpose and the nature of this optional component.
- I have taken reasonable steps to ascertain the identity of the participant or the participant's legal representative signing this Information & Consent Form for Future Research.
- I have taken reasonable steps to ascertain that the participant or the participant's legal representative has not been coerced into giving the consent.

Witnessed by: _____ _____
Name of witness Date of signing

_____
Signature of witness

1. An impartial witness (who is 21 years of age or older, has mental capacity, who is independent of the research study, and cannot be unfairly influenced by people involved with the research study) should be present during the entire informed consent discussion if a participant or the participant's legal representative is unable to read, and/or sign and date on the consent form (i.e. using the participant's or legal representative's thumbprint). After the written consent form and any written information to be provided to participant, is read and explained to the participant or the participant's legal representative, and after the participant or the participant's legal representative has orally consented to the participant's participation in the study and, if capable of doing so, has signed and personally dated the consent form, the witness should sign and personally date the consent form. This is applicable for Clinical Trials regulated by HSA and Human Biomedical Research under the HBRA.

2. For HBRA studies, the witness may be a member of the team carrying out the research only if a participant or the participant's legal representative is able to read, sign and date on the consent form.

Investigator's Statement

I, the undersigned, certify to the best of my knowledge that the participant/ participant's legal representative signing this Information & Consent Form for Future Research had the optional component (storage of data for future use in other research studies) fully explained to him/her and clearly understands the purpose and the nature of this optional component.

_____	_____	_____
Name of Investigator/ Person obtaining consent	Signature	Date

Appendix 2: Instructions for ultrasound of rectus femoris muscle

A. Ensure the patient is lying comfortably, with leg extended in neutral position. The head of bed should ideally be inclined at 30 degrees.

B. Locating position
   - Choose the right leg wherever possible. Use the same leg for measurements throughout the study.
   - Locate the base of the iliac crest and the top of the patella. Measure the distance and mark the mid-point (children < 6 years) or 1/3 the distance from the patella (children >6 years).

C. Ultrasound measurement
   1. Use the linear probe with the largest footprint available.
   2. Ensure that the settings are correct. Suggested standardized settings are a frequency of 12.0MHz, Gain of 50 and Dynamic Range (DR) of 95. Ensure that that the time-gain is in the neutral position.
   3. Adjust settings if necessary, between patients. Ideally the image should be as large as possible, while allowing visualization of the skin surface as well as the bone. For each patient, the following settings should remain the same
      i. Depth
      ii. Gain
      iii. Frequency
   4. Create a new exam
      i. Enter in patient ID
      ii. When the rectus femoris can be visualized appropriately, press "freeze" and then save picture.
      iii. For the cross-sectional ultrasound measurement, ensure that there is copious gel and minimal compression of the skin.
      iv. Label image with subject ID, location, scan no. etc. Suggest to record as: SubjectID_location at leg_timepoint of measurement_image number. E.g. ID01_1/2RL_1_3 (this shows subject 1, measured at ½ of right leg, first measurement, image 3.
      v. Press "freeze" again to unfreeze pane, and repeat.
   5. Capture 3 images and save each image. Name each image appropriately.
   6. Export the DICOM images.

D. Measuring the cross-sectional area
   1. Using the appropriate software with DICOM format support (e.g. NIH ImageJ tool), draw the cross-sectional area by tracing the inner echoic edge of the rectus femoris cross sectional area.
   2. Record the cross-sectional area in cm^2

Appendix Table 1. Functional status scale score by Pollack et al. 2009

Domains	Normal (Score = 1)	Mild Dysfunction (Score = 2)	Moderate Dysfunction (Score = 3)	Severe Dysfunction (Score = 4)	Very Severe Dysfunction (Score = 5)
Mental status	Normal sleep/wake periods; appropriate responsiveness	Sleepy but arousable to noise/ touch/ movement and/or periods of social non-responsiveness	Lethargic and/or irritable	Minimal arousal to stimuli (stupor)	Unresponsive, coma, and/or vegetative state
Sensory functioning	Intact hearing and vision and responsive to touch	Suspected hearing or vision loss	Not reactive to auditory stimuli or to visual stimuli	Not reactive to auditory stimuli and to visual stimuli	Abnormal responses to pain or touch
Communication	Appropriate non-crying vocalizations, interactive facial expressiveness, or gestures	Diminished vocalization, facial expression, and/or social responsiveness	Absence of attention getting behavior	No demonstration of discomfort	Absence of communication
Motor functioning	Coordinated body movements, normal muscle control, and awareness of action and reason	1 limb functionally impaired	≥2 limbs functionally impaired	Poor head control	Diffuse spasticity, paralysis, or decerebrate/ decorticate posturing
Feeding	All food taken by mouth with age-appropriate help	Nothing by mouth or need for age-inappropriate help with feeding	Oral and tube feedings	Parenteral nutrition with oral or tube feedings	All parenteral nutrition
Respiratory status	Room air and no artificial support or aids	Oxygen treatment and/or suctioning	Tracheostomy	Continuous positive airway pressure treatment for all or part of the day and/ or mechanical ventilatory support for part of the day	Mechanical ventilator support for all of the day and night

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	10
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	10
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	3
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	4

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	7

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	4
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	6

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	6
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	8
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	2
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	2
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA