Statistical Analysis Plan for a multi-centre randomised controlled trial: The effect of early intravenous amino acid supplementation in critically ill patients with normal kidney function

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on behalf of the ESSENTIAL Trial Investigators

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Change log from Ver 1 to Ver 1a.
- update ChiCTR number (Page 1)
- update study web site address (Section 1.4, Page 4)
- correct mistranslation of Inclusion Criteria to reflect study web site (Appendix 1, Page 13)
- adjust table border that previously obscured Exclusion Criteria 11 and 12 and reformat to fit on one page (Appendix 1, Page 14).

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Contents

BRIEF BACKGROUND

1.1 Overview:

Guided by the findings of a published subgroup analysis,[1] we initiated and conducted a multi-centre, Phase III, randomised controlled trial (RCT) to determine if the provision of an early, continuous, supplementary, infusion of a standard mixture of L-amino acids improves survival from critical illness in patients with normal renal function.

1.2 Hypotheses to be tested:

In critically ill patients with normal kidney function, does an early, continuous, supplementary infusion of a standard mixture of L-amino acids alter all-cause 90-day landmark mortality compared to standard care?

1.3 Eligibility criteria:

See **Appendix 1** for the complete list of study eligibility criteria, to be applied within the first two days of study ICU admission.

1.4 Randomisation:

Allocation concealment was maintained through the use of a central randomisation web site that was secure, encrypted and password protected.

The study website was accessible 24 hours a day, seven days a week. This academic website (https:// https://capctg.medbit.cn/en/2022/01/04/essential/) has been used to host numerous secure research projects.

The randomisation sequence was generated using SAS Version 9.4 with blocks of variable size and random seeds [2] to ensure allocation concealment could not be violated by guessing the allocation sequence at the end of each block. Randomisation was stratified within study site. Stratification variables and their thresholds were concealed from site investigators to further prevent anticipation of the allocation sequence.[3]

1.5 Study intervention:

If randomised to the intervention arm, the patient received a continuous infusion of a standard mixture of L-amino acids (18AA-VII, traumatic amino acid, Haisco, China) delivered at a rate to achieve a total daily protein intake of approximately 2.0 g/kg/day.

The initial infusion was begun at approximately 1 g/kg/day. If the patient was receiving any form of enteral or parenteral nutritional support, the infusion rate of the study L-amino acid intervention (18AA-VII, traumatic amino acid, Haisco, China) was reduced such that the total protein intake from all sources (nutrition and study intervention) was approximately 2.0g/kg/day.

The study intervention was discontinued at discharge from the study ICU, or death, or when the patient's central venous catheter was removed.

Study web tool and amino acid infusion protocol (Mini Program on the Wechat platform)

The study web tool calculated study amino acid infusion rates based on a patient's current protein intake from Enteral and Parenteral nutrition and the patient's weight. Protein intake calculations for overweight (BMI > 25 kg/m^2) patients were based on their ideal body weight (ideal BMI set at 23 kg/m^2).

The majority of patients commenced study amino acid at an infusion rate of 42 ml/h, which provided 100g protein per day.

The protocol reduced the study amino acid infusion to a lower rate **only** if total protein from EN, PN and study amino acid reached 2.5g/kg.

If total protein from EN, PN and study amino acid infusion reached 2.5g/kg, the Protocol reduced the patient's study amino acid infusion rate such that a total protein intake of 2.0g/kg from EN, PN and study amino acid was achieved.

The study web tool was used to conduct all calculations. Results were printed or written on to a blank form to create a permanent record of calculations. It was the site investigator's responsibility to ensure that the appropriate study amino acid infusion rates, as calculated by the study web tool, were effectively communicated to, and achieved by, the bedside health care team.

If a patient switched to a different brand of EN or a different type of PN, or if EN or PN was started or discontinued, the study web tool was used to calculate a new study amino acid infusion rate.

The study web tool was not password protected. It could be accessed by the bedside nurse on the weekend / at night if required.

1.6 Standard care:

Standard care in the patients randomised to the control arm consisted of a reasonable attempt to provide enteral or parenteral nutrition when the attending clinician judged the patient would tolerate feeding. The attending clinician selected the route, starting rate, metabolic targets, and protein goals based on current practice in their ICU.

1.7 Data collection and follow up:

Every randomised patient was followed up until 90 days post-randomisation, unless death occurred first, as recommended by the UK Medical Research Council International Working Party for Clinical Trials in Patients with Sepsis and Septic Shock.[4] If patients remained in hospital on study Day 90, follow-up was censored and outcomes were recorded as per status at Day 90.

1.8 Summary of study outcomes:

The primary study outcome was defined as patient vital status (alive/dead) to be determined on the 90th calendar day post-randomisation.

Secondary outcomes consisted of the following: Duration of ICU stay; Duration of invasive mechanical ventilation; Duration of Hospital stay; Death in ICU; Death in hospital; Survival duration; Days alive out of hospital; Incidence of clinically significant renal dysfunction (proportion of patients per study arm); Days of Renal Replacement Therapy (RRT); Incidence of RRT (proportion of patients per study arm); continuing need for RRT at Study Day 90 (proportion of patients per study arm); Days of clinically significant organ failure (reported by organ system); New onset organ failure; New receipt of organ support.

Process measures were collected to describe the implementation of the study intervention (study amino acid infusion). These process measures included: time from ICU admission to feeding start; time from study enrolment to feeding start; number of days of study amino acid infusion in patients allocated to receive the study intervention; mean nutrition support days per 10 patient days in patients receiving EN and or PN; mean nutrition support days per 10 patient days in patients receiving EN; mean nutrition support days per 10 patient days in patients receiving PN; percent of patients who were never fed; percent of patients fed within 24h of ICU admission; Mean energy (not including study amino acid infusion) delivered in kcal/patient-day; Mean energy (including energy from study amino acid infusion) delivered in kcal/patient-day; Mean total protein delivered in g/kg/patient-day; Mean energy delivered per patient for each of the first seven days of ICU stay; Mean protein delivered per patient for each of the first seven days of ICU stay.

Secondary outcomes	**Definitions and/or analysis approach**
Duration of ICU stay	in days
Duration of invasive mechanical ventilation	in days
Duration of Hospital stay	in days
Death in ICU	proportion of patients per study arm
Death in hospital	proportion of patients per study arm
Days alive out of hospital	in days
Incidence of clinically significant renal	proportion of patients per study arm

ysfunction	
Days of Renal Replacement Therapy (RRT)	in days
Incidence of RRT	proportion of patients per study arm
Continuing need for RRT at Study Day 90	proportion of patients per study arm
Days of clinically significant organ failure	in days
New onset organ failure	proportion of patients per study arm
New receipt of organ support	proportion of patients per study arm

Process measures	**Definitions and/or analysis approach**
Time from ICU admission to feeding start	in days
Time from study enrolment to feeding start	in days
Number of days of Supplementary Protein in patients allocated to receive the study intervention	in days
Mean nutrition support days per 10 patient days in patients receiving EN and or PN	in days
Mean nutrition support days per 10 patient days in patients receiving EN	in days
Mean nutrition support days per 10 patient days in patients receiving PN	in days
Percent of patients who were never fed	proportion of patients per study arm
Percent of patients fed within 24h of ICU admission	proportion of patients per study arm
Mean energy (not including study amino acid) delivered in kcal/patient-day	in kcal/patient-day
Mean energy (including energy from study amino acid) delivered in kcal/patient-day	in kcal/patient-day
Mean total protein delivered in g/kg/patient-day	in g/kg/patient-day
Mean energy delivered per patient for each of the first seven days of ICU stay	in kcal/patient-day
Mean protein delivered per patient for each of the first seven days of ICU stay	in kcal/patient-day

STATISTICAL ANALYSIS

2.1 Safety and Data Monitoring Committee:

An independent Data and Safety Monitoring board (DSMB), comprising experts in clinical trials, biostatistics and intensive care was established. The committee reviewed information on all serious adverse events.

Using the Haybittle-Peto approach,[5;6] the DSMB was charged with informing the study management committee if there was a difference in serious adverse events between study groups that exceeded three standard deviations in magnitude.

2.2 Sample size and power:

Conservative estimates of potential effect size were obtained from a previously published formal subgroup analysis demonstrating an early, continuous, supplementary, infusion of a standard mixture of L-amino acids may significantly reduce mortality (P=0.034, 7.9% absolute risk difference [ARD], 0.65 relative risk reduction [RRR]).[1]

Given best estimates of a baseline mortality rate of 15.9% in standard care patients,[7] and assuming the relative risk reduction (0.65) is preserved, using standard formulas [8] a trial of 1,838 patients (919 per group) would have 90% power to detect an absolute risk difference of 5.5%. A trial of this size (N=1,838) also provided 80% power to detect a treatment effect as low as 4.8% ARD and, in the worst case scenario, if the

trial yielded a treatment effect as low as 3.4% ARD, the overall findings could still be expected to be statistically significant at the traditional two-sided P-value threshold of 0.05.

2.3 Basic principles of analysis: Modified intention to treat efficacy analysis

With the release of the EFFORT Protein trial in January 2023,[9] an Interim Safety Meeting of ESSENTIAL Trial Investigators was held in March 2023. At this meeting it was discussed that EFFORT Protein reported strong signals that patients with Acute Kidney Injury at study baseline may be harmed by higher protein intake (Relative risk of mortality was increased by 1.4 times, 95% Confidence Interval 1.1 to 1.8, EFFORT Protein Supplementary Appendix Page 16). Based on this information, it was deemed prudent to alter the ESSENTIAL Trial's eligibility criteria to exclude patients with AKI at time of enrolment. (See Appendix 1) Therefore, after March 4, 2023, no patients with AKI were enrolled.

The primary conclusions of this project will be based on a modified intention to treat analysis for efficacy which will focus on all randomised patients with normal baseline renal function (Ex. do not have AKI), regardless of whether they actually received the intended treatment or whether a protocol violation or protocol deviation occurred. Safety analyses will be based on all randomised patients. Sensitivity analysis including all randomised patients (Ex. enrolled with AKI before Protocol Change) will be conducted to support inferences drawn from the primary modified intention to treat efficacy analysis.

Patients who withdrew consent for use of their data will not be included in any analysis. Only the facts that they were enrolled into the trial and withdrew consent, and the original study group to which they were allocated, will be reported.

Two-sided 5% significance levels will be used to identify statistically significant results. A two-sided 10% significance level will be used to identify results that are trending towards statistical significance. All confidence intervals reported will be 95% confidence intervals.

Adjustments for multiplicity will not be undertaken because a hierarchy of outcomes has been stipulated [10] and because the conduct of an interim analysis using Haybittle-Peto stopping thresholds does not require adjustment of outcomes for multiplicity.[5;6]

2.4 Primary outcome:

The primary study outcome is defined as patient vital status (alive/dead) to be determined on the 90th calendar day post-randomisation.

2.5 Missing primary outcomes:

Missing primary outcomes (Day 90 vital status) will be assumed to be missing at random (MAR) and thus will be 'ignored' in the primary analysis [11] however if greater than 1% of all primary outcomes that should be available for analysis are missing, a sensitivity analysis will be undertaken in addition to the primary MAR analysis.

This sensitivity analysis will include an evaluation of the results of models developed under the assumptions of: 1) last observation carried forward; 2) worst extreme case imputation and; 3) regression model imputation using all available information.[12,13] Results of the primary MAR analysis will be interpreted in the context of these sensitivity analyses.

Information that is *unavailable* for analysis due to withdrawal of consent for data use will not be considered *missing* and therefore will not be included in the estimate of percent missing as described above nor will it be included in a simulation study.

2.6 Unadjusted analysis of primary outcome:

The unadjusted analysis of the effect of treatment on the primary outcome will be assessed using an exact Pearson chi-square test. This test will not be adjusted for continuity.

The magnitude of the treatment effect will be reported as an odds ratio with exact precision-based 95% confidence intervals and a risk difference with exact test-based 95% confidence intervals.

2.7 Distribution of baseline prognostic variables (aka Manuscript Table 1):

The following baseline prognostic variables, ascertained at time of study enrolment, will be reported by study group in Manuscript Table 1:

Age, Gender, BMI (as a continuous variable *and* categorised into percent underweight [BMI < 18] and not underweight [BMI ≥ 18]), APACHE II score, APACHE II defined presence of Acute Renal Failure, Source of admission to ICU (ED, OR, ward, ICU readmit, Other hospital), Surgical admission type (Elective, Emergency), Chronic Health States (Hepatic cirrhosis, Chronic dialysis, Respiratory Disease, CV Disease, Immunocompromised), APACHE III admission dx major category (Cardiovascular/vascular, Respiratory, Trauma, GI, Neuro, Sepsis, Metabolic, Haematological, Other surgical, Other medical), need for invasive mechanical ventilation, and measures of organ dysfunction defined as per SOFA score. Receipt of the following agents within 24h prior to enrolment: a distal loop diuretic; acetazolamide; nephrotoxic agents and; nephrotoxic pigments. Recent history of: obstructive uropathy; oliguria and; massive transfusion. Pre-acute illness history of abnormal kidney function.

Continuous variables, which are expected to be Normally distributed, will be presented as Mean and Standard Deviation. Dichotomous variables will be presented as Numerator/Denominator and Percent.

Manuscript Table 1 will not present p-values however variables identified as meeting objective pre-established criteria for inclusion in a *covariate adjusted analysis* of the primary outcome will be marked with an asterix (*) or hash (#). The asterix (*) will denote variables that are shown to be strongly associated with outcome, which may confound even in the presence of minimal imbalance. The hash (#) will denote variables shown to have strong imbalance, which may confound even in the presence of a weaker association with outcome (See section 2.9 for complete details).

2.8 Missing baseline prognostic variables:

Exclusion of randomised patients with known outcomes from analysis, for any reason, contravenes the intention to treat principle.[14] Every effort should be made to minimise post-randomisation exclusions.[11]

By default, statistical software packages require complete information on all covariates for a patient case to be included in a covariate adjusted regression model. Any missing information from any covariate results in the exclusion of the entire patient case by the software package. Exclusion of incomplete cases with known outcomes reduces statistical efficiency and introduces bias into the estimate of treatment effectiveness.[15;16;17]

Missing baseline prognostic variables will be replaced with mean values calculated from the observed non-missing instances of that baseline prognostic variable.[18] The imputed means will be calculated using pooled data from both treatment arms. Imputed means will *not* be calculated within treatment arm using treatment arm-specific data *nor* will any post-randomisation information be incorporated into the calculation. Furthermore, replacement values for missing calculated constructs such as BMI and APACHE II score will be estimated using non-missing component-level information. For example, if one of the components of BMI is missing, such as height, overall mean height will be imputed and BMI will be calculated with the known weight and imputed mean height.

If a baseline prognostic variable requires imputation of missing values, the percent of cases that were originally missing will be reported.

2.9 Covariate adjusted analysis of primary outcome:

A *covariate adjusted analysis* of the effect of treatment on the primary outcome will be undertaken. An objective pre-specified algorithm will be used to select variables for inclusion in the covariate adjusted Poisson (or negative-binomial) regression model.[19] The primary purpose of the covariate adjusted analysis will be to remove bias from the estimate of the treatment effect on the primary outcome.

All prognostic variables reported in Manuscript Table 1 (see Section 2.7) will be eligible for inclusion in the covariate adjusted analysis. Neither a *centre effect term* nor *any interaction terms* will be considered in the covariate adjusted model.[19]

Step 1: *Identification of prognostic variables with a strong association with outcome*
Prognostic variables shown to be strongly associated with outcome, even if not shown to be imbalanced between treatment groups, will be screened for inclusion in the covariate adjusted model as they may remove bias from the estimate of treatment effect.[19;20;21;22]

Univariate logistic regression analysis will be conducted to evaluate the relationship between each prognostic variable identified in Section 2.7 *and the study primary outcome*.

Prognostic variables with a Likelihood Ratio Test (LRT) p-value less than or equal to 0.15 will qualify for evaluation in the *maximum covariate adjusted model* (see Step 3).[23] Inferences will not be drawn from the interpretation of this univariate p-value, the p-value will simply be used to describe the *strength* of association between the prognostic variable and the primary outcome.[21]

Step 2: *Identification of prognostic variables with strong imbalance between treatment groups*
Prognostic variables shown to be strongly imbalanced between treatment groups, even if associations with outcome are shown to be weak, will be screened for inclusion in the covariate adjusted model as they may remove bias from the estimate of treatment effect.[19;20;21]

Univariate logistic regression analysis will be conducted to evaluate the relationship between each prognostic variable identified in Section 2.7 *and allocated treatment group*.

Prognostic variables with a LRT p-value less than or equal to 0.15 will qualify for evaluation in the *maximum covariate adjusted model* (see Step 3).[23] Inferences will not be drawn from the interpretation of this p-value, the p-value will simply be used to describe the *strength* of imbalance between treatment groups.[21]

We acknowledge that simulation studies demonstrate the addition of this step *may not* improve performance over 'predictor' detection alone (Step 1) however these simulations are not definitive.[22] 'Imbalance' detection *may* help preserve the face validity of the covariate adjusted results [24] and *may* remove bias from the estimate of treatment effect.[23;24]

Step 3: *Backwards stepwise elimination from the maximum model*
Parsimony must be embraced during the development of a covariate adjusted *Poisson* regression model because covariate adjustment may not always increase precision in the way that would be expected in a *least-squares* regression model for a continuous outcome.[19] Indeed, if a covariate does not reduce bias in the estimate of treatment effect, there may be no practical gains from its inclusion in a covariate adjusted *Poisson* regression model.[23]

All prognostic variables identified by Step 1 and Step 2 will be included in a *maximum covariate adjusted regression model.* The treatment group term (Amino Acid Supplementation vs. standard care) will be forced to stay in the maximum model. The model outcome will be the study primary outcome.

If the maximum model demonstrates issues arising due to collinearity based on Eigenanalysis and Condition Number, collinearity will be addressed by standardizing and scaling of continuous variables before backwards stepwise elimination begins.[25]

Step 3a: Prognostic variables will be eliminated from the maximum model, one variable at each step, if their multivariate LRT p-value is greater than 0.10.

Step 3b: From the subset of prognostic variables remaining after Step 3a, prognostic variables will be eliminated from the maximum model, one variable at each step, if their multivariate impact on bias in the estimate of the treatment effect is negligible.[26;27] A negligible impact will be defined as less than 5% change

in the regression coefficient for the treatment effect after stepwise removal of the prognostic variable from the subset model.[22]

Step 4: *Final covariate adjusted model*
The *final* covariate adjusted model will contain all prognostic variables known to have a meaningful impact on bias in the estimate of the treatment effect as identified by the execution of Steps 1 to 3 (above). The complete *final* model will be presented as the *covariate adjusted model* in the primary paper.

The LRT p-value for the estimate of treatment effect from this model will be reported.

6.0 *A priori* defined subgroup analysis:

A priori identified subgroup analysis will be conducted on the following baseline variables: Age (>59 vs. ⩽ 59), BMI (>25 vs. ⩽25), SOFA (⩾9 vs. <9)，and SGA muscle wasting (well nourished vs. other categories).

Screening for differential subgroup treatment effects on the primary study outcome will be conducted using a formal test of interaction. The p-value for this interaction term will be obtained from an LRT.

The logistic regression model will contain a main effect term denoting the specific subgroup of interest, a main effect term for treatment group and a subgroup × treatment interaction term. If the two-sided LRT p-value for this test of the subgroup × treatment interaction term is less than 0.10, the presence of differential treatment effects within subgroups will be reported in the primary publication along with the LRT p-value for the interaction term.

Detailed subgroup analysis will be undertaken *only* within subgroups identified to have differential treatment responses by the screening process described above. Detailed subgroup analysis will adhere to the same analytic principles and plan outlined for the overall study results. Detailed subgroup analysis will include reassessment of the baseline distribution of prognostic variables within the subgroup of interest, development of a subgroup appropriate covariate adjusted model and reassessment of all study outcomes within the subgroup. The results of any detailed subgroup analysis will be reported in subsequent papers, to be submitted for publication soon after the submission of the primary publication.

The number of *a priori* subgroup analyses (**4**) will be reported in all publications. Due to the use of conservative tests of interaction to screen for the need to conduct detailed analysis within subgroups, no corrections to p-values will be undertaken for multiple-comparisons.

6.1 Exploratory, hypothesis generating subgroup analyses:

No hypothesis generating subgroup analyses, including efficacy subset type analyses,[11] will be undertaken for, or reported in, the primary publication.

However, the study protocol does report an intent to undertake exploratory analyses to determine if any demographic or physiological measures recorded at entry into the trial (Section 2.7) can identify patient groups most likely to benefit from treatment in order to inform the design of any subsequent clinical trials. These will be reported in subsequent publications.

If any hypothesis generating subgroup analyses are reported in subsequent publications, they will be clearly identified as hypothesis generating when reported.

The number of *a priori* subgroup analyses will be reported in all publications along with the total number of any *hypothesis generating* subgroup analyses previously undertaken.

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APPENDIX 1: Detailed Study Inclusion and Exclusion Criteria

Inclusion Criteria

Patients will be considered ***eligible*** for the trial if ***all*** of the following *inclusion criteria* are met at the time of screening:

1. Informed consent form obtained from the patient or next of kin;
2. 18 years old or older;
3. Within 48h of ICU admission;
4. Expected to stay in ICU for more than 2 days (Ex. Not expected to be discharged on day after enrolment.);
5. Have a working central venous access line through which the study intervention could be delivered;
6. Be able to tolerate at least 1L of fluid volume per day.
7. APACHEII score ≥ 15 or SOFA score ≥ 6.

See next page for Exclusion Criteria.

Exclusion Criteria

Patients will be considered ***ineligible*** for the trial if ***any*** of the following *exclusion criteria* are met at the time of screening: (Answer **NO** to all questions)

1.Patients is currently receiving an selective COX-2 inhibitors;

2.Patients receiving palliative treatment or expected to die within 48 hours;

3.**{Before Protocol change** March 4, 2023**}** Have severe Acute Kidney Injury, defined as: current serum creatinine (SCr) increased 3 times pre-acute illness value OR SCr > 350 μmol/L with recent increase greater than 44 μmol/L. [Note: If pre-acute illness creatinine values are unknown, assume upper limit of normal: 90 μmol/L for females and 110 μmol/L for males.]

3. **{After protocol change** March 4, 2023**}** Have Acute Kidney Injury, defined as: current serum creatinine (SCr) increased 1.5 times pre-acute illness value. [Note: If pre-acute illness creatinine values are unknown, assume upper limit of normal: 90 μmol/L for females and 110 μmol/L for males.]

4.Patients with malignant diseases receiving radiotherapy or chemotherapy;

5.Currently receiving or scheduled for dialysis/renal replacement therapy;

6.Patients ever had a kidney transplant;

7.Patients require treatment of a burn injury to greater than 20% of total body surface area;

8.Patients have a documented contraindication to the study intervention (IV amino acids);

9.Known to be pregnant or currently breastfeeding;

10.Have severe liver disease (Biopsy proven cirrhosis, or documented portal hypertension with a known past history of either upper GI bleeding attributed to portal hypertension or of hepatic failure leading to encephalopathy / coma);

11.Have a documented hypersensitivity (known allergy) to one or more of the included amino acids;

12.Have a documented inborn error of amino acid metabolism.