Statistical Analysis Plan:

Supplemental enteral protein in critically ill trauma and surgical patients: A randomized clinical trial

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1. Trial Synopsis

The aim of this study is to determine the effect of enteral protein supplementation on biochemical measures of inflammation and protein metabolism in critically ill surgical patients. The investigators will also collect data on important clinical outcomes, including infectious complications, duration of mechanical ventilation and other measures of recovery from critical illness.

Overall Hypothesis: That early supplemental enteral protein will increase serum concentrations of transthyretin at three weeks after the onset of illness or injury. Secondarily, we will test whether supplementation increases ventilator-free days and reduces ventilator-associated pneumonia.

2. Primary Objective

Overall Aim: To determine the effect of enteral protein supplementation on serum protein markers, ventilator-free days, and complications in critically ill trauma victims.

Primary Hypothesis: That early supplemental protein will increase serum concentrations of transthyretin at two weeks after injury.

Secondary hypothesis: that supplementation improves clinical outcomes shown by an increase in ventilator free days (reduced duration of mechanical ventilation).

3. Design

The trial will use an open label parallel randomized trial design with a planned 500 patients receiving enteral nutrition randomized 1:1 to 2 g/kg/day of protein vs standard care.

4. Primary Estimand Statement[1]

Population: critically ill adult trauma patients who are started on enteral nutrition within 72 hours after injury and are expected to require nutritional support for at least 1 week. Non-trauma post-surgical patients meeting study entry criteria will be included as well. This is expected to include patients recovering from emergency surgery, including amputations, debridement of necrotizing soft-tissue infections, and other serious surgical illnesses.

Treatments: Enteral protein with a target rate of 2 g/kg/day vs standard enteral nutrition.

Variable of Interest: Circulating transthyretin at 3 weeks (14-21 days after injury)

Primary efficacy analysis population definition: All randomized participants

Intercurrent events: Subjects may be missing transthyretin due to death, discharge from the hospital, or recovery. Those participants who die will have a transthyretin level equal to the lowest value observed amongst the surviving subjects. Participants who are discharged alive

from the hospital before the primary outcome is measured will have a transthyretin level set equal to the highest observed measure. Multiple imputation will be used to account for study participants who did not have transthyretin measured in the expected window.

Summary measure: The difference in mean transthyretin levels between treatment arms.

5. Study Population

The investigation will enroll 500 subjects using the following inclusion and exclusion criteria:

Inclusion: critically ill adult trauma patients who are started on enteral nutrition within 72 hours of admission to the intensive care unit and are expected to require nutritional support for at least 1 week. Non-trauma post-surgical patients meeting study entry criteria will be included as well. This is expected to include patients recovering from emergency surgery, including amputations, debridement of necrotizing soft-tissue infections, and other serious surgical illnesses.

Exclusion: An individual who meets *any* of the following criteria will be excluded from participation in this study:

- Significant chronic organ failure,
- severe malnutrition,
- not expected to survive due to severity of injuries and,
- serum creatinine >2 mg/dl.

6. Data sources

Data will be collected concurrently with study subject enrollment and treatment in the intensive care unit. extracted from the electronic medical record.

7. Outcomes

7.1 Primary endpoint:

Circulating transthyretin concentrations measured 14 – 21 days after injury.

7.2 Secondary endpoint:

- Ventilator free days: the numbers of days on which the subject does not require assisted ventilation during the first 28 days following the date of injury. VFD will be set to zero for all participants who die before hospital discharge.
- Hospital acquired pneumonia: Pneumonia diagnosed while the patient is in the hospital. Pneumonia will be defined as the presence of a positive quantitative culture from a bronchoalveolar lavage sample coupled with the use of antibiotics.

7.3 Subgroups for primary endpoint

- NUTRIC score (< 5 vs ≥ 5),
- CRP (> 100 vs $<= 100$),

- CRP (> 100 vs <= 100) at day 14 (or closest measurement) in those participants in the ICU at least 7 days,
- Trauma patients only.

7.5 Exploratory outcomes:
- ICU-free days in the first 28 days after injury. ICU-free days will be set to zero for those participants who die before hospital discharge.
- Death before hospital discharge.
- Time to hospital discharge alive.
- Pneumonia from the trauma registry for trauma patients.

7.6 Safety outcomes:
- Select adverse events, including gastrointestinal intolerance and possible aspiration.
- Dialysis.
- Blood urea nitrogen.
- Serum creatinine.

8. Statistical Methodology

8.1 Randomization

Participating patients were randomized to high (an additional 2 g/kg/day) vs. standard protein levels in a ratio of 1:1.

8.4 Intent to Treat (ITT) Analysis

All efficacy analysis will be conducted under the ITT principle. Patients will be included in the study arm according to the treatment group to which they were randomly assigned. All randomized subjects will be included in these analyses.

8.5 Primary Statistical Hypothesis

The null hypothesis is that the mean circulating transthyretin level, measured between days 14 and 21, in the group of subjects randomized to the intervention arm is equal to the mean in the control group.

8.6 Primary Analysis

A t-test with unequal variances will be used to test the primary hypothesis. The analysis will use 20 imputed data sets, with results combined across imputations.

8.7 Secondary Analysis

Analyses of the VFD endpoint will be based on the intent-to-treat principle using imputed data if there is missing outcome data. The mean VFD for the increased protein group will be compared to the mean within the control group using a t-test allowing for unequal variances.

The number and proportion of participants diagnosed with hospital acquired pneumonia will be summarized. The proportion will be compared between treatment arms using a score-based test.

8.8 Subgroup Analyses

The primary analysis will be repeated in subgroups defined by:

- mNUTRIC score (<5 vs ≥5),
- CRP (> 100 vs <= 100),
- CRP (> 100 vs <= 100) at day 14 (+/-) in those participants in the ICU at least 7 days; and,
- Trauma patients only.

8.9 Exploratory Analyses

Analyses of the ICU-free days endpoint will be based on the intent-to-treat principle using imputed data if there is missing outcome data. The mean ICU-free days for the increased protein group will be compared to the mean within the control group using a t-test allowing for unequal variances. The proportion of participants dying before discharge will be summarized by treatment arm and compared using a score-based test for equality of proportions. The time to discharge alive, with death as a competing risk, will be assessed using cumulative incidence functions.

The proportion of participants with trauma who have a diagnosis of pneumonia noted in the trauma registry will be summarized by treatment arm and compared using a score-based test for equality of proportions. To assess the association of transthyretin with VFD, a linear regression analysis will be performed.

The rate of increase in transthyretin will be compared between the two treatment groups using a GEE model to account for the correlation of observations within each study participant. The model will use the identity link and working independence, and covariates for study day, treatment arm, and the interaction of treatment with study day. This exploratory analysis will include all study participants with any transthyretin measures available.

8.10 Safety Analyses

8.10.1 Analyses of Adverse Events

The number and proportion of subjects reporting key adverse events will be summarized for both treatment groups.

For both treatment groups, we will report the average, standard deviation, median, and quartiles of creatinine. The maximum value reported during each of the first four weeks of hospitalization will be summarized (Days 1-7; Days 8-14; Days 15-21; Days 22-28).

8.10.2 Other Safety Analyses

The number and proportion of subjects receiving dialysis during their hospitalization will be summarized for both treatment groups.

8.11 Multiplicity

In this study we will use an alpha level of 0.05 for the primary and secondary comparisons. For exploratory and safety analyses, confidence intervals but not p-values will be provided.

8.12 Missing Data and Sensitivity Analyses

The primary and secondary analyses will be performed by multiple imputations to alleviate, as much as possible, the impact of any missing data (death, loss to follow-up, or participant withdrawals) on the analysis of those outcomes. Estimates across the 20 imputations will be combined using Rubin's approach (Rubin 1989). Additionally, sensitivity analyses will be performed using a tipping point analysis. The tipping point analysis will investigate how extreme the values of the missing data must be to change the conclusions of the trial (if that is possible).

8.12.1 Multiple Imputation

Twenty complete imputed datasets will be created using linear regression. The multiple imputation model will be performed within each treatment group for those who survive hospital discharge, and within deaths separately. Covariates will include age, sex, race/ethnicity, NUTRIC score, ventilator free days, and previous values of transthyretin when available. For each complete dataset, the differences in means between treatment groups as well as the standard error of that difference will be calculated; these results will be combined across imputations using Rubin's rule.[2]

8.12.2 Tipping Point Analysis

A tipping point analysis will be used to explore the sensitivity of the results to the missing at random assumption (MAR) used in the main multiple imputation model. A range of treatment effects in those missing outcome data will be considered by adding a shift parameter to the multiple imputation mode described above. This shift parameter, δ, will be applied only to study participants in the treatment group, and thus create a systematic difference in the missing outcomes across arms beyond what is observed in the data. For each value of δ, multiple imputed data sets will be created and analyzed as above. The results of these analyses will allow us to calculate the change in the mean transthyretin in those missing the primary outcome that would alter the conclusion of the primary study results.

Last Observation Carried Forward
As a sensitivity analysis, we will analyze the data using the last observation carried forward for those subjects with missing primary outcome data.

9. Sample Size Calculations / Power

With 500 participants this study has 80% power to detect a difference of 2.4 mg/dl in average circulating transthyretin levels during the third week between the protein supplementation group (assumed average circulating transthyretin level of 14.4, and the standard of care group (assumed average circulating transthyretin level of 12.0, with a pooled standard deviation of 7.0). This conservatively assumes that there will be 25% of the patients for whom the protein supplementation has no effect in that they are either well enough to have high circulating transthyretin levels or die prior to obtaining the primary outcome. Because the primary outcome is a surrogate marker, we do not plan for any formal interim analyses to evaluate efficacy or futility. Safety of our intervention will be reviewed by an independent Data Safety Monitoring Board (DSMB) on a regular basis. Currently, the Harborview Medical Center typically admits about 300 - 350 trauma victims per year who would meet eligibility criteria. With enrollment over 4 - 5 years, we anticipate that ~1500 patients will be eligible. Therefore, the rate of recruitment among eligible patients needs to be ~33%. The statistical analysis of the primary hypothesis will use a two-sided t-test with alpha of 0.05. We expect that less than 10% of patients will die prior to day 21. For these and for patients who are lost-to-follow-up prior to day 21, we will multiply impute the primary outcome separately for each group and incorporate previously observed circulating transthyretin levels (if available) and patient age information as well as sex, race/ethnicity, NUTRIC score, and ventilator free days.

10. Statistical and Data Programming

All data cleaning and programming will be done in either the statistical software R (R Foundation) or in SAS (SAS).

References (for this document: Statistical Design and Power)

1. Pohl M, Baumann L, Behnisch R, Kirchner M, Krisam J, Sander A. Estimands-A Basic Element for Clinical Trials. *Dtsch Arztebl Int*. Dec 27 2021;118(51-52):883-888. doi:10.3238/arztebl.m2021.0373
2. Rubin DB. *Multiple imputation for nonresponse in surveys*. Wiley series in probability and mathematical statistics Applied probability and statistics,. Wiley; 1987:xxix, 258 pages.