

# Evaluation the effects of pycnogenol (French maritime pine bark extract) supplementation on the inflammatory biomarkers, nutritional and clinical status in traumatic brain injury patients, in Intensive Care Unit; A Randomized Clinical Trial protocol

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## Study protocol

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# Abstract

**Background:** Traumatic brain injury (TBI) is one of the major health and socioeconomic problems in the world. Immune-enhancing enteral formula has been proven to significantly reduce infection rate in TBI patient. One of the ingredients that can be used in immunonutrition formulas to reduce inflammation and oxidative stress is pycnogenol. **Objective:** surveying the effect of pycnogenol on the clinical, nutritional and inflammatory status of TBI patients. **Methods:** This is double-blind, randomized controlled trial . Block randomization will be used. Intervention group will receive pycnogenol supplementation of 150 mg for 10 days. Control group will receive placebo for the same duration. Inflammatory status (IL-6, IL- 1 $\beta$ , C-reactive protein) and oxidative stress status (Malondialdehyde, total antioxidant capacity), at the base line, at the 5 th day and at the end of the study (10 th day) will be measured. Clinical and nutritional status will be assessed three times during the intervention. SOFA (sequential organ failure assessment) questionnaire for assessment of organ failure will be filled out every other day. The mortality rate will be calculated within 28 days of the start of the intervention. Weight, body mass index and body composition will be measured. All analyses will be conducted by initially assigned study arm in an intention-to-treat analysis. **Discussion:** We expect that supplementation of 150 mg pycnogenol for 10 days will improve clinical and nutritional status and reduce the inflammation and oxidative stress of the TBI patients.

## Background

### Traumatic brain injury

Traumatic brain injury (TBI) is one of the major health and socioeconomic problems in the world (1). It is prevalent in both developed and developing societies and affects people of all ages. TBI is called the 'silent epidemic' because problems resulting from TBI does not occur immediately (2). TBI causes about 1.5 million deaths and hospitalizations per year in the USA (3). TBI is more common among teens and young adult aged 15-45 years, mainly due to road accidents and sport-related events. Men are three times more likely to be injured and more severely damaged (4).

TBI is used instead of 'head injury' because it shows the importance of the 'brain' (5). TBI is defined as: 'An alteration in brain function or physiology due to external force or shock from the outside (6). Patients with TBI are categorized into three groups based on Glasgow Coma Scale: mild, moderate, and severe. Glasgow Coma Scale (GCS) is a system used to assess coma and impaired consciousness (7). A GCS score of 13–15 is defined as mild, 9–12 as moderate, and 3–8 as severe (7).

The mechanisms of damage to the brain tissue associated with TBI is classified into two categories: primary and secondary. Primary damage is due to the mechanical force involved in the skull and the brain, which seems to be irreversible (8). The primary injury complications include: brain contusions, axonal injuries, rupturing of blood vessels, and intracranial hemorrhages. Secondary injury complications progress over time (9). The secondary injury complications include: elevated intracranial pressure, blood-brain barrier (BBB) disruption, neuroinflammation, brain edema, cerebral hypoxia, ischemia, and delayed neurodegeneration (10-12)

## Neuroinflammation in TBI

Cytokines, chemokines, and growth factors have proven to play important roles in the pathophysiology of TBI. Immediately after brain injury, Proinflammatory cytokines, such as IL-1 $\beta$ , IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) as well as transforming growth factor-beta (TGF- $\beta$ ) are produced in large volume. These worsen the condition of trauma and delay the recovery by producing oxidative stress and Matrix metalloproteinases (13, 14). These post-traumatic inflammatory cascades cause blood brain barrier (BBB) dysfunction, which ultimately leads to the influx of inflammatory cells from the blood to the brain (15).

Production of reactive oxygen substrates (ROS) directly or indirectly, as oxidative by-products of lipids, proteins, or nucleic acids are common following traumatic brain injury. Malondialdehyde (MDA) are the major by-products of lipid peroxidation. MDA is potentially atherogenic lipid peroxide and generated in vivo via peroxidation of polyunsaturated fatty acids (16).

## Nutrition support in TBI

Nutritional support is an important issue in intensive care for critically ill patients such as those with TBI. Patients with TBI often remain in a hypermetabolic state where the energy expenditure is increased (17). Early nutrition support in TBI patients result in a significant reduction in mortality rate, less infectious complications and lower risk of poor outcome (18). There has been growing use of immunonutrition to modulate the inflammatory response in injury or infection and to improve clinical outcomes (19). Immune-modulation enteral formula has been proven to significantly reduce infection rate in TBI patients (20). One of the ingredients that can be used in immunonutrition formula to reduce inflammation and oxidative stress is pycnogenol.

## Pycnogenol

Pycnogenol® (PYC) is recognized as one of the most powerful natural antioxidants, which is a bark extract of the French maritime pine (*Pinus pinaster*) and is rich in flavonoids. The main component of PYC are: polyphenols, specifically mono- and oligomeric units of caffeic acid, ferulic acid, catechin, epicatechin, and taxifolin (21). It is classified as GRAS (generally recognized as safe) in the USA (22). Clinical effects of PYC include: endothelium-dependent vasodilator activity (23), and anti-thrombotic effect as shown by numerous in vitro and in vivo investigations in animals and human clinical research studies (24, 25). PYC prevents neurotoxicity and apoptotic cell death in oxidative stress status (26, 27). Also, PYC protects against lipid peroxidation, and pro-oxidants and peroxy nitrates (28, 29). A number of animal studies proved the protective effect of PYC following traumatic brain injury by suppressing of IL-6 and TNF- $\alpha$  level (30, 31). No serious adverse effect has been seen in any clinical trials or commercial use (32). The most commonly observed adverse effect is gastric discomfort due to mild and transient nature (22).

Recently, we conducted a systematic review and meta-analysis of clinical trials that used PYC in chronic diseases (33). Our meta-analysis revealed that PYC supplementation may have beneficial effects on glycolipid metabolism by reducing fasting glucose, HbA1c, LDL and enhancing HDL. Also, PYC reduced CRP, plasma free radicals, systolic and diastolic blood pressure and body mass index. In this study, for the first time in the world we aimed to assay the PYC effect on inflammatory markers and clinical status in acute phase in humans.

## **Objectives**

### **Main objective**

Previous human studies reported the neuroprotective and anti-inflammatory effect of PYC. The effects of PYC to reduce neuroinflammation in TBI rat has been proved. So the main objective of present study is to study the effect of PYC on the clinical, nutritional and inflammatory status of TBI patients as the first human study in the world.

### **Specific objectives**

In achieving this overall goal, numerous issues will be addressed with the specific objective of providing definitive answers to the following questions: 1) Is PYC effective in reducing the inflammatory markers including IL-6, IL-1 $\beta$  and CRP (C-reactive protein) in TBI patients in intensive care unit? 2) Is PYC effective in reducing oxidative stress in TBI patients? 3) Is PYC effective in improving clinical status of TBI patients, by improving APACHE II (acute physiologic and chronic health evaluation II) (supplementary table 1) and SOFA (sequential organ failure assessment) (supplementary table 2) score? 4) Is PYC effective in improving anthropometric indices (weight and body composition) and nutritional score measured via Nutric score questionnaire? 5) Is PYC effective in reducing 28-day mortality in TBI patients?

### **Trial design**

This is a parallel group randomized trial. Blocked randomization will be used to allocate eligible participants to either the control group or the intervention group. The study framework is superiority.

## **Methods/design**

### **Patient selection**

### **Inclusion criteria**

All TBI patients admitted directly or transferred to the intensive care units of participating hospital will be evaluated for eligibility for entry into the randomized clinical trial. Preliminary eligibility criteria are summarized in Table 1.

### **Exclusion criteria**

Patients meeting all preliminary eligibility criteria are considered potentially eligible for the study. Patients are screened for the presence of any specific exclusion criteria which would preclude study entry. These exclusions are designed to eliminate patients for whom participation may be dangerous or patients with serious medical disorders whose impact on operative outcome may obscure the importance of nutritional, clinical and inflammatory factors. These are summarized in Table 2.

### **Default criteria**

Once randomization has taken place, patients will be removed from the study only for the following reasons: 1) patient's or physician's request, 2) significant change in patient's treatment process, 3) create any exclusion criteria, 4) sensitivity to PYC supplementation.

### **Sample size**

Sample size calculations were based upon Luzzi et al's study (34) which showed the mean CRP change in the treatment group was 60% and in the control was unchanged or up to a maximum of 15%. Based on the formula for comparing two proportions of a qualitative attribute from two independent statistical societies, the sample size was determined as 25 individuals in each group, ( $\alpha= 0.05$ ,  $\beta=0.1$ , the power of study is 90%). Assuming a probable drop out the sample, 30 patients in each group will be considered.

A study evaluating the effect of PYC on IL-6 and TNF- $\alpha$  in TBI was an animal study (30) and inappropriate for the human sample size calculation. Therefore, we used CRP as an inflammatory factor to calculate the sample size. However, for more certainty, we calculated the sample size according to Hakumat Rai et al study (35) based on IL-6 change in TBI patients. Based on the mean difference between the two groups for IL-6 and error level of 5% and power of 85% the total sample size calculated was 46. With a 10% probability of drop out during the study, the total sample size was 50 (25 in each group).

### **Study procedures**

The university's executive committee will oversee the project's implementation and progress, information security, safety of trial participants, and scientific impact assessment. Also this committee will review data from the trial. The trial sponsor will undertake auditing of the trial procedure.

#### **Randomization and masking**

We will randomly allocate eligible patients on enrolment (1:1) to either the control group or the intervention group. The randomization list of unique patient identifiers is generated by the computer-generated random block size site. The classification is based on age (18 to 40 and 40 to 65 years old), gender (male / female) and APACHEII score (0 to 35 and 35 to 71) using quadruple blocks.

Nutritionists or clinicians will keep the sealed opaque envelope containing the unique patient identifier and the study group allocation in a locked cabinet in the study laboratory. They will be opened by the

second nutritionist. Investigators, all study staff hospital attending clinical teams, and patients will be masked to the study group allocation.

## **Intervention**

A pragmatic (36), parallel-group, double-blind, randomized controlled trial (table 3) will be conducted. We will enroll 60 patients who are admitted to ICU at a university hospital in Tehran, Iran. All participants or their first-degree relatives will need to provide informed consent to the clinician before participating.

Participants will be randomly divided in two groups. The method of randomization and masking are explained above. At the first visit, baseline data will be gathered and the intervention group will receive PYC supplement (OLIGOPIN) in the form oral capsules containing 50 mg French maritime pine bark extract plus 130 mg Microcrystalline Cellulose. OLIGOPIN powder of each capsule will be dissolved in 10 ml deionized water and given to patients via gavage (3 capsule per day) for 10 days.

Control group will receive oral capsules containing 130 mg Microcrystalline Cellulose with 10 ml of deionized water via gavage (3 capsule per day) for 10 days.

The capsules will be given by the investigator to the patients by gavage, so fidelity to the intervention will be strong. However for more certainty, at the end of each day, the number of capsules remaining for each patient will be checked. In order to control the confounding effect of food intake, both the control group and the intervention group receive the standard formulas based on their daily required energy via enteral root feeding.

## **Possible risk assessment of intervention**

Initially an intervention with a dose of 150 mg of PYC will be started for 10 patients, and in the absence of clinical complications and observing the expected effect on the reduction of inflammatory markers, the same dose will be continued. Otherwise, it will be reduced to 100 mg, if there is any adverse effect. There have been no reports of serious adverse event in any clinical trials or commercial use of OLIGOPIN. However, these patients will be regularly evaluated biochemically and clinically each day, and the liver function tests including serum levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) will be checked. If there are any potential complications from intervention or, if the physician determines that the intervention should be discontinued, the supplements will be immediately removed from the patient's enteral nutrition.

## **Data collection**

Data will be collected at four main times: at the base line, at 5th day of intervention, at 10th day of intervention and at the 28-day follow-up visit. At the base line demographic characteristics are gathered via a questionnaire. Anthropometric assessment including height (via ulna length), weight (by using portable scale "Balas"), body mass index and body composition (by using bio impedance device "Inbody") will be measured at the base line, 5th day and at the end of intervention.

In order to evaluate inflammatory and oxidative stress markers, 10 cc of venous blood will be taken from each patient at the base line, at the 5th day and at the end of the study. The serum sample will be isolated and used to measure the markers via ELISA kits. APACHE II (for assessment of clinical status of patients) and Nutric questionnaires (for assessment of nutritional status) will be filled out at the base line, 5th day and the end of study. SOFA questionnaire (for assessment of organ failure) will be filled out every other day. The mortality rate will be asked by phone within 28 days of the start of the intervention. A SPIRIT diagram of the recommended content for the schedule of enrolment, interventions, and assessments is shown in figure 1.

## **Data management**

Specially designed forms will be completed by study staff at each time point, and scanned, verified and committed to a local site database within 48 h of completion. Completed forms will be stored as the source documentation in a locked cabinet, with access restricted to specified study team members. The forms will be identified by a unique participant ID number and will not contain any patient identifiable information. Queries based on data in the database will be generated daily, including date, range and logic checks.

## **Outcomes**

The measurable outcomes are summarized in table 4.

## **Statistical methods**

The trial profile will be summarized using a CONSORT flow chart, including reasons for non-eligibility and non-enrolment (37). The objective of this clinical trial is to determine if PYC supplementation improves clinical and nutritional outcomes in TBI patients admitted in ICU or not. To answer this question, the outcomes of patients receiving PYC supplement will be compared with the outcomes of patients receiving placebo.

All analyses will be conducted by initially assigned study arm in an intention-to-treat analysis, and adjusted for randomization site. Thus, all randomized patients who will receive at least one dose of study treatment and who will have both a baseline and at least one post baseline measurement will be analyzed. The data will be expressed as mean  $\pm$  SD. Statistical analyses will be conducted with SPSS version 19 (SPSS Institute, Chicago, Ill). Chi-square test will be done for categorical variables. T test will be done to assess the statistical significance of the continuous variables. Comparable nonparametric test (Mann-Whitney U test) will be substituted when tests for normality and equal variance failed. A value of P 0.05 will be used as a criterion for statistical significance. Survival analysis will be performed with log-rank test. The study design flow diagram is summarized in figure 2.

More details about the statistical analyses plan is presented in appendix.

## Discussion

The purpose of this trial is to establish proof of concept of the efficacy of Oligopin in patients with TBI (GCS score  $\geq$  8). Patients will be screened and randomly enrolled into the intervention and control groups based on age, gender and APACHEII score. Adding Oligopin to nutritional formula might reduce neuroinflammation and oxidative stress and improve clinical and nutritional status in TBI patients. But human study about PYC (Oligopin) in this patient group has not been done so far.

It is assumed, that oxidative stress mediated through the superoxide radical (superoxide) and other reactive oxygen species (ROS) may be principal to inflammation and impaired neural function (38). The acute inflammatory response differ in early and late stages of TBI; too much inflammation for too long delays recovery (39). Shortly after brain injury, there is mass production of proinflammatory cytokines, such as IL-1 $\beta$ , IL-6 and CRP (40).

In the event of TBI, IL-1 $\beta$  is the most studied cytokine. Glial cells produce IL-1 $\beta$  and affects neurons and other brain cells. IL-1 $\beta$  motivates inflammatory responses and aggregates immune cells, disrupts the BBB and forms edema, and leads to loss of neurons (41). The high level of IL-1 $\beta$  has been detected in CSF and brain tissue within early hours of brain injury in humans as well as in experimental animals (41). Administration of anti-IL-1 $\beta$  antibodies decreased edema and degradation of brain tissue. A previous study reported improvement of cognitive function in rats following TBI (42). There are similar findings for IL-6. Intervention to mitigate IL-6 in animals with mild TBI, triggers normal brain function and reduces the effects of hypoxia (aggravation of inflammation of brain damage) (43). In TBI patients, CRP levels are correlated with the duration of hospitalization in ICU and dependence on the ventilator, and the severity of the damage (44). Finally, we selected these inflammatory factors as outcomes of the study.

### Duration of intervention

In this study we selected 10 days for intervention. According to previous studies, the odds of survival in the first 10 days of admission of the patients in the ICU have a declining slope and after 10 days the slope of the decline will be milder (46, 47). Therefore, any intervention of treatment in this period (the first 10 days), which leads to a reduction in the risk of mortality, has great importance. On the other hand, the duration of intervention used in clinical trials to evaluate the clinical effects of PYC supplementation has varied from several hours to several months (22, 48, 49). So in this study we expect to see the expected effects after 10 days.

### Dose of supplementation

The average dose used in most human studies that has beneficial effects in improving inflammation is 150 milligrams (22, 50, 51). No side effects have been reported in this dose. Therefore we chose 150 mg Oligopin for this study.

### Trial status

This trial is registered at [clinicaltrials.gov](https://clinicaltrials.gov) (ref: NCT03777683) at December 17, 2018 and is on going. It is the first version of the protocol. In April 2019 recruitment began, and anticipated date to complete the study is February 2020.

## Abbreviations

**TBI:** Traumatic brain injury

**PYC:** pycnogenol

**APACHE II:** The Acute Physiology and Chronic Health Evaluation

**SOFA:** The Sequential Organ Failure Assessment

**Nutric score:** Nutrition assessment in critically ill

**ALT:** alanine aminotransferase

**AST:** aspartate aminotransferase

**CRP:** C-reactive protein

**IL-1 $\beta$ :** Interleukin-1 $\beta$

**IL-6:** Interleukin-6

**MDA:** Malondialdehyde

**TAC:** Total antioxidant capacity

**ROS:** Reactive oxygen substrates

**TGF- $\beta$ :** Transforming growth factor-beta

**TNF- $\alpha$ :** Tumor necrosis factor- $\alpha$

**BBB:** Blood- brain barrier

## Declarations

### Ethics approval and consent to participate

Central ethical approval has been confirmed from the Research Ethics Committees of the Mashhad University of medical sciences (ref approval no. IR.MUMS.MEDICAL.REC.1397.460) and we will not begin recruiting at other centers in the trial until local ethical approval has been obtained". All study procedures

were in accordance with the ethical standards of the Helsinki Declaration. The informed consent will be obtained from all study participants and their guardians.

### **Availability of data and materials**

Final study datasets will be stored locally and securely at Trauma and Injury Research Center, Iran University of medical sciences, Tehran, Iran for long-term storage and access. Participant level data will be made available by request on a case-by-case basis.

All Principal Investigators will access to the data sets. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

### **Funding**

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### **Author contribution**

Malekahmadi M and Norouzy A designed research protocol; *Malekahmadi M and Moradi Moghaddam O will* conduct research; Malekahmadi M , Pahlavani N, and Firouzi S wrote the protocol; Tanha K designed the statistical plan. Norouzy A, Nematy M, Islam SMS, Zali M: modified the final protocol. Norouzy A had primary responsibility for final content. All authors read and approved the final protocol manuscript.

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## Tables

Table 1. primary criteria for study eligibility

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Admission in ICU <sup>a</sup> due to TBI <sup>b</sup>
18 year ≤ age ≤ 65 year
GCS <sup>c</sup> score ≥ 8
Stable hemodynamic and metabolic status in the first 24 to 48 hours
Having enteral nutritional support
Fill out the informed consent form by the patient or first-degree relatives of the patient

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<sup>a</sup> Intensive care unit. <sup>b</sup> Traumatic brain injury. <sup>c</sup> Glasgow coma scale.

Table 2. exclusion criteria

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Pregnancy and lactation
Morbid obesity: BMI <sup>a</sup> ≥ 40
Failure to start enteral nutrition in the first 24-48 hours
Suffering from autoimmune disorders and HIV/Aids
Suffering or having History of cancer and any liver failure
Receiving positive inotropic medications including Dopamine, Dobutamine and Epinephrine
Severe and active bleeding
Suffering from Sepsis
Having history of known food allergies

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<sup>a</sup> Body mass index.

Table 3. Nine PRECIS-2 domains for trial designing characteristics

Domain	Considerations
Eligibility	All eligible TBI <sup>a</sup> patients admitted directly or transferred to the ICU <sup>b</sup> of participating hospital will take PYC <sup>c</sup> Along with their routine treatment.
Recruitment Path	Pragmatic recruitment through usual visit of patients in ICU.
Setting	All tests and evaluation status of patients in this trials are performed routinely as a part of usual care, except specialized test including: IL-6 <sup>d</sup> , IL-1 $\beta$ <sup>e</sup> , MDA <sup>f</sup> and TAC <sup>g</sup> .
Organization	ICU that has intensivist and its trained staff
Flexibility in Delivery	PYC will be prescribed with gavage.
Flexibility in Adherence	After the written consent of the patient or his / her companion, PYC will be prescribed by the intensivist and will be gavaged by the nurse. PYC is a dietary supplement and it does not interfere with other medications and will be given as part of the patient's nutritional formula.
Follow-up	Usual follow up that performed for critically ill patients will be done in this trial also 28 days follow up for mortality will be performed.
Primary Outcome	All outcome directly are related to clinical status of patients.
Primary Analysis	Intention-to-treat with all available data will be used for analysis.

<sup>a</sup> Traumatic brain injury. <sup>b</sup> Intensive care unite. <sup>c</sup> Pycnogenol. <sup>d</sup> Interleukin-6. <sup>e</sup> Interleukin-1 $\beta$ . <sup>f</sup> Malondialdehyde. <sup>g</sup> Total antioxidant capacity

Table 4. measurable outcomes

outcome	Time Frame	Measurement method
Change of inflammatory markers: IL-6 <sup>a</sup> , IL-1 $\beta$ <sup>b</sup>	5 and 10 days	ELISA kit
Change of inflammatory marker: CRP <sup>c</sup>	5 and 10 days	Auto analyzer
Change of oxidative stress markers: MDA <sup>d</sup> , TAC <sup>e</sup>	5 and 10 days	Calorimetry*
Change of weight	5 and 10 days	Portable scale "Balas"
Change of body fat percentage	5 and 10 days	Bio impedance device "Inbody"
Change of body mass index	5 and 10 days	Equation
Change of APACHEII <sup>f</sup> score	5 and 10 days	APACHEII score questionnaire
Change of SOFA <sup>g</sup> score	3, 5, 7, 9, 10 day	SOFA score questionnaire
Change of Nutric score	5 and 10 days	Nutric score questionnaire
28-day mortality	28 days	Telephone follow up

<sup>a</sup> Interleukin-6. <sup>b</sup> Interleukin-1 $\beta$ . <sup>c</sup> C reactive protein. <sup>d</sup> Malondialdehyde. <sup>e</sup> Total antioxidant capacity <sup>f</sup> acute physiologic and chronic health evaluation II. <sup>g</sup> sequential organ failure assessment.

\*Colorimetry is a scientific technique that is used to determine the concentration of colored compounds in solutions by the application of the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance.

## Figures

TIMEPOINT**	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
	-24h to 48h	0	Day1	Day 3	Day 5	Day 7	Day 10	Day 28
<b>ENROLMENT:</b>								
Eligibility screen	X							
Informed consent	X							
medical history	X							
Allocation		X						
<b>INTERVENTIONS:</b>								
Intervention pycnogenol			←————→					
Intervention placebo			←————→					
<b>ASSESSMENTS:</b>								
inflammatory markers			X		X		X	
anti- inflammatory marker			X		X		X	
oxidative stress markers			X		X		X	
weight			X		X		X	
body fat percentage			X		X		X	
body mass index			X		X		X	
APCHEII <sup>a</sup> score			X		X		X	
SOFA <sup>b</sup> score			X	X	X	X	X	
Nutric score			X		X		X	
28-day mortality								X

<sup>a</sup> acute physiologic and chronic health evaluation II. <sup>b</sup> sequential organ failure assessment.

Figure 1

SPIRIT diagram of recommended content for the schedule of enrolment, interventions, and assessments

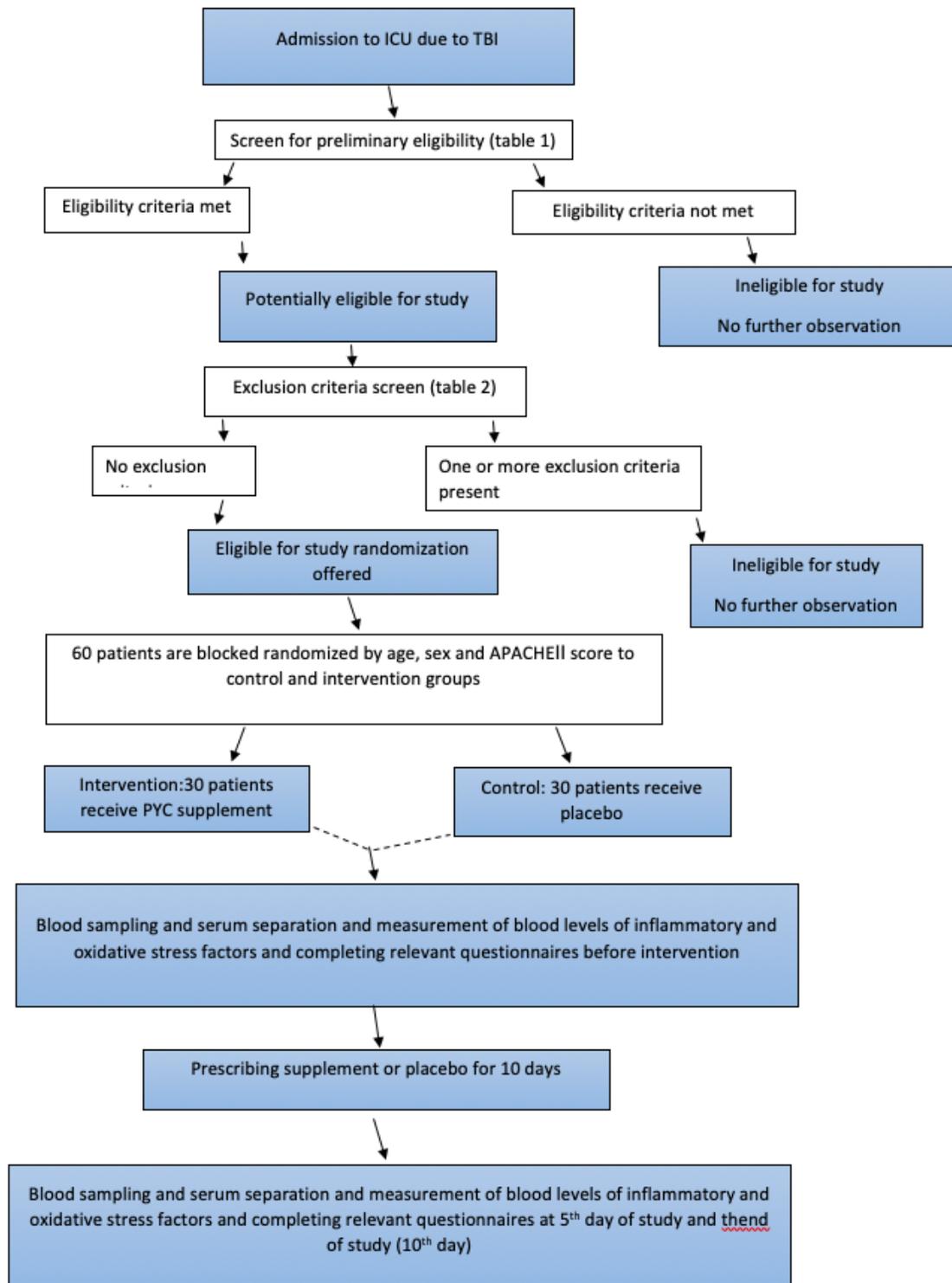


Figure 2

Study design flow diagram

## Supplementary Files

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- [appendixSAP.pdf](#)
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