The effect of ulinastatin on sepsis-related organ failure in children: a multicenter randomized controlled trial (The IMPROVING Study)

Rationale

Sepsis is a clinical syndrome caused by infection and is one of the leading causes of death in critically ill children. According to the latest data, there are about 48 cases of sepsis and 22 cases of severe sepsis per 100,000 children. The mortality of sepsis ranges from 7.1 to 28.5%, and the mortality of children with multiple organ dysfunction syndrome (MODS) is as high as 33.5%[1]. Studies have shown that endothelial damage due to sepsis is an important mechanism for exacerbation and the development of MODS [2]. Endothelial cell damage leads to increased vascular permeability, which allows fluid to enter tissues and cause volume depletion[3]. In addition, vascular endothelial injury promotes platelet activation and thrombosis, aggravating tissue ischemia and injury [4]. This series of pathophysiological processes ultimately leads to the exacerbation of the condition and the occurrence of MODS [5]. Therefore, the protection and repair of endothelial cells has become one of the important strategies for the therapy of sepsis.

In recent years, with the growing awareness of the importance of immune system response and its interaction with vascular endothelial cells, there has been increasing attention to endothelial cell injury in sepsis caused by inflammatory mediators. In sepsis, the releases of cytokines and chemokines can act directly on endothelial cells, causing damage. In addition, inflammatory mediators promote adhesion between leukocytes and endothelial cells, a process that leads to mechanical damage to endothelial cells and activation of intracellular signaling pathways, which in turn leads to further exacerbation of the inflammatory response. Under the action of inflammatory mediators, endothelial

cells are further promoted to undergo apoptosis, and the apoptotic endothelial cells are shed, which will further exacerbate the increase of vascular permeability [6-8]. The above evidence suggests that inflammatory mediator intervention and monitoring have important evaluation value for the occurrence and development of endothelial cell injury in sepsis.

Ulinastatin (UTI) is a serine protease inhibitor found in human blood and urine, which is part of the innate immunity of human, with broad-spectrum anti-inflammatory, regulating immunity, improving microcirculation, and some studies have found that in patients with severe sepsis and septic shock, the application of ulinastatin is related to survival benefits [9]. A large number of clinical pharmacodynamic studies have shown that UTI can inhibit the excessive release of inflammatory mediators and lysosomal enzymes, reduce the level of inflammatory factors, protect endothelial cells, regulate microcirculation and immune function, and reduce the mortality of patients with shock [10-13]. UTI can significantly improve capillary permeability, improve microcirculation[11], significantly reduce the damage of sepsis to organs such as lungs, liver and kidneys, and curb the development of MODS. Unfortunately, these are all animal-level and single-centre small studies, and the evidence is not strong enough and needs to be further confirmed by prospective, multicenter study, especially for children.

In order to better evaluate the efficacy of ulinastatin therapy, it is necessary to find a target for endothelial injury based on the pathophysiological basis of MODS due to sepsis. At present, in clinical practice, the methods used to evaluate endothelial cell damage include in vivo evaluation, imaging examination, and monitoring of peripheral blood markers, among which the monitoring of peripheral blood markers is simple and easy to implement in clinical practice [14, 15]。Thrombomodulin (TM) is one of the main markers reflecting the degree of endothelial damage[16-19]，and has also been reported to be closely related to the prognosis of sepsis. Therefore, this study aimed to evaluate ulinastatin based on inflammatory factors and endothelial cell damage to improve the organ damage and prognosis of sepsis, so as to

provide an effective means to block the occurrence of sepsis-related MODS.

Methods

Study Design

This is a multicenter, prospective, Interventional study performed in 8 children's hospitals from China. Children participating in this study will be randomly assigned to one of two groups. The first group will receive ulinastatin through an intravenous (IV) drip every 8 hours for 7 days. The dosage will be adjusted based on the child's weight but will not exceed a certain limit. The second group will receive an equal amount of normal saline (a harmless fluid often used in medical treatments) as a placebo.

Participants

All patients meet the inclusion criteria at the participating sites will be recruited. We will collect patients consecutively admitted to each participating center within a-month period. Every site needs to initiate a "run-in" phase before formally recruiting patients after ethics approval. The "run-in" phase should include at least 5 patients to refine study process and ensure data quality in the following formal study. All the data collected during the "run-in" phase will not be included for analysis. Formal recruitment will commence in the next calendar month after the "run-in" phase. Informed consent is required for each participant and/or the guardian of the participant in this study, either signed by the patient himself and/or the guardian of the patient. All the data stored in the electronic database are de-identified to guarantee patients privacy.

Inclusion criteria

1. 28 days < age ≤ 18 years

2. There is clear evidence of infection

3. Meet diagnostic criteria for sepsis: infection + pSOFA and/or Phoenix Sepsis Score (PSS) ≥2 for each organ.

Exclusion criteria

1. Received immunomodulatory therapy in 2 months before enrollment, such as Xuebijing and thymosin;

2. Use of high-dose non-steroidal anti-inflammatory drugs within two days before enrollment; The daily dose of glucocorticoids (equivalent to methylprednisolone) > 5 mg/kg or greater than 500 mg/day;

3. Abandonment of treatment of the patient or disagreement with aggressive life support treatment;

4. Treatment with ulinastatin prior to enrollment;

5. Previous history of allergy to ulinastatin or any of its components;

6. Children with terminal disease.

Withdrawal of participants

Participants will be withdrawn from the clinical research study in the following circumstances:

1. In cases of withdrawal of informed consent, where capacity exists.
2. In cases of withdrawal of consent by the participant's guardian for children with incapacity.

Participants may withdraw from the trial at any time during the trial. Withdrawal from the trial will not result in fines, discrimination, or retaliation, nor will it affect your future medical treatment and rights. If the participant wish to withdraw from the trial, the participant should inform the study physician promptly. The study physician will ensure that you are able to end this trial in the safest way.

Data collection and management

A web-based electrical database will be used for data collection and storage. All data will be de-identified with unique ID and input by the primary investigator or nominated investigators (less than two for each participating center) approved by the primary investigator, and a double check will be done by the research coordinator. Training for data entry will be performed by the provider of the electrical database (Nanjing Origin of Life Medical Technology Co., Ltd, Nanjing, China). According to the schedule shown in Table 1, the investigator will collect data during the first seven days of enrollment or before discharge, which ever happen first, and follow up the patients at day 28 after enrollment for key clinical outcomes like ICU stays and 28-d mortality.

The principal investigator's center will be responsible for data safety, privacy, and quality. The data will be monitored regularly for data quality control by a data management team at the principal investigator's center.

Outcome measurements

Primary outcome measure:

Composite outcome of 28-day mortality and/or presence of at least one organ failure on day 7 after randomization (pSOFA ≥2 and/or Phoenix Sepsis Score≥2 for each organ system)

Secondary outcome measures:

① Thrombomodulin levels on day0, day1, day 2 and day 7 of randomization.

② Length of ICU stay to day 28 after randomization.

③ Days of survival without life-support interventions to day 28 after randomization.

④ Cytokine levels on day 0, day 1, day 2 and day 7 of randomization.

⑤ pSOFA and/or Phoenix Sepsis Score scores on day 0, day 1, day 2 and day 7 of randomization.

⑥ Blood lactate levels on day 0, day 1, day 2 and day 7 of randomization.

⑦ WBC, CRP, PCT, LDH levels on day 0, day 1, day 2 and day 7 of randomization.

⑧ 24-hour fluid output and intake within the first 7 days of randomization.

⑨ Cumulative use of steroids within the first 7 days of randomization (equivalent to methylprednisolone).

Planned Statistical Analysis

SAS V9.4 software was used for all statistical processing. All statistical tests were performed using two-sided tests, and $P<0.05$ was defined

as statistically significant, with 95% confidence intervals (CIs).

The normality of the continuous data was analyzed by the Kolmogorov-Smirnov test, and if the data followed a normal distribution, the results were expressed as mean ± standard deviations, and the comparison between the two groups was analyzed by independent t-test. If the data do not follow a normal distribution, they are presented as medians (quartiles) and comparisons between the two groups were performed using the Mann-Whitney U test. The count data was presented with the number and percentage of cases in each category, and the chi-square test was used to compare the rates between the two groups, and the Fisher exact probability test was used if necessary.

Comparisons of primary and secondary measures were analyzed using a generalized linear mixed-effects model (with centers as a random effect) to derive effect sizes and 95% confidence intervals for the intervention group relative to the control group. For 28-day mortality, the K-M survival curve was plotted according to the time-event data, the p-value was obtained by log-rank test, and the hazard raito (HR) and 95% confidence interval were estimated by Cox proportional hazards regression.

Subgroup analyses will be performed in two categories: different age (age ≥ 5 vs. age < 5), sex (Male or Female), body mass index (> 85th percentile of BMI of the same age, sex and ethnicity vs ≤85th percentile of BMI of the same age, sex and ethnicity), presence or absence of septic shock (Yes vs No).

Ethics approval and dissemination

This study was approved by the ethics committee of Children's Hospital of Soochow University. The ethical approval document ID is 2024018. Even when central ethical approval has been confirmed, we will not begin recruiting at other participating centers in the trial until the local ethics

committee approved the study. The results will be published in research articles or conference papers.

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Table 1. Schedule of enrollment, assessment and follow up.

	Study period			
	Enrollment	**Observational period**		**Follow up**
Time point		Day0[a]	Day1-Day7	Day28
Enrollment:				
Eligibility screen	X			
Informed consent	X			
Demographic information	X			

Assessment:				
Fluid intake and outflow per day			⟷	
Organ failure		X	⟷	
Laboratory test		X	⟷	
Organ support therapy		X	⟷	
Steroid therapy			⟷	
Follow up:				
28-d mortality				X
ICU days				X

a: Day0 is defined as the day from enrollment to 8 am the next day.