The Effects of Limited Infusion Rate of Fluid in the Early Resuscitation of Sepsis on Syndecan-1 Shedding: a Randomized Controlled Trial (LIFE3S trial)

Miss Jutamas Saoraya

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Clinical Sciences
Common Course
FACULTY OF MEDICINE
Chulalongkorn University
Academic Year 2020

ผลของการให้สารน้ำจำกัดความเร็วในการกู้ชีวิตผู้ป่วยพิษเหตุติดเชื้อต่อซินดิแคน-1:การศึกษาแบบสุ่มและมีกลุ่มควบคุม

น.ส.จุฑามาศ เศารยะ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเวชศาสตร์คลินิก ไม่สังกัดภาควิชา/เทียบเท่า
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	The Effects of Limited Infusion Rate of Fluid in the Early Resuscitation of Sepsis on Syndecan-1 Shedding: a Randomized Controlled Trial (LIFE3S trial)
By	Miss Jutamas Saoraya
Field of Study	Clinical Sciences
Thesis Advisor	Associate Professor KHRONGWONG MUSIKATAVORN, M.D.
Thesis Co Advisor	Associate Professor NATTACHAI SRISAWAT, M.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the FACULTY OF MEDICINE
(Professor SUTTIPONG WACHARASINDHU, M.D.)

DISSERTATION COMMITTEE

..... Chairman
(Assistant Professor Opass Putchareon, M.D.)
..... Thesis Advisor
(Associate Professor KHRONGWONG MUSIKATAVORN, M.D.)
..... Thesis Co-Advisor
(Associate Professor NATTACHAI SRISAWAT, M.D.)
..... Examiner
(Professor Rujipat Samransamraujkit, M.D.)
..... Examiner
(Assistant Professor Sahadol Poonyathawon, M.D.)
..... External Examiner
(Associate Professor Suthat Rungruanghiranya, M.D.)

จุฑามาศ เศารยะ : ผลของการให้สารน้ำจำกัดความเร็วในการกู้ชีวิตผู้ป่วยพิษเหตุติดเชื้อต่อซินดิแคน-1:การศึกษาแบบสุ่มและมีกลุ่มควบคุม. (The Effects of Limited Infusion Rate of Fluid in the Early Resuscitation of Sepsis on Syndecan-1 Shedding: a Randomized Controlled Trial (LIFE3S trial)) อ.ที่ปรึกษาหลัก : รศ. นพ.ครองวงศ์ มุสิกถาวร, อ.ที่ปรึกษาร่วม : รศ. นพ.ณัฐชัย ศรีสวัสดิ์

ความเป็นมา การรักษาด้วยการให้สารน้ำอย่างรวดเร็วเป็นการรักษาที่แนะนำในการกู้ชีพผู้ป่วยภาวะพิษเหตุติดเชื้อ ทั้งนี้อาจทำให้มีผลเสียเนื่องจากมีการทำลายของชั้นไกลโคแคลิกซ์ งานวิจัยนี้ต้องการศึกษาผลของการให้สารน้ำจำกัดความเร็วต่อการบาดเจ็บของไกลโคแคลิกซ์ที่วัดด้วยซินดิแคน-1 ในผู้ป่วยพิษเหตุติดเชื้อที่มีการไหลเวียนบกพร่อง วิธีการศึกษา ทำการศึกษาแบบสุ่มและมีกลุ่มควบคุม ในเดือนพฤศจิกายน พ.ศ. 2561 ถึงกุมภาพันธ์ พ.ศ. 2563 ในห้องฉุกเฉินที่เป็นสถาบันฝึกสอนแห่งหนึ่ง ผู้ป่วยพิษเหตุติดเชื้อที่มีการไหลเวียนบกพร่องซึ่งนิยามว่ามีความดันโลหิตต่ำหรือมีค่าแลคเตทสูงถูกสุ่มเพื่อได้รับสารน้ำความเร็วมาตรฐาน (30 มิลลิลิตรต่อกิโลกรัมต่อชั่วโมง) หรือสารน้ำจำกัดความเร็ว (10 มิลลิลิตรต่อกิโลกรัมต่อชั่วโมง) ในช่วง 30 มิลลิลิตรต่อกิโลกรัมแรก ผลลัพธ์หลักคือการเปลี่ยนแปลงค่าซินดิแคน-1ในพลาสมาในช่วง 6 ชั่วโมง ผลลัพธ์รองคือเหตุการณ์ไม่พึงประสงค์ ภาวะอวัยวะล้มเหลว และการเสียชีวิตใน 90 วัน ผลการศึกษา ทำการวิเคราะห์ผู้ป่วย 96 คนในงานวิจัยโดยมีผู้ป่วยกลุ่มละ 48 คน ค่ามัธยฐานของสารน้ำใน 6 ชั่วโมงในกลุ่มจำกัดความเร็วเท่ากับ 39 มิลลิลิตรต่อกิโลกรัม (ค่าพิสัยระหว่างควอร์ไทล์ 35-52 มิลลิลิตรต่อกิโลกรัม) เทียบกับส่วนในกลุ่มความเร็วมาตรฐานเท่ากับ 53 มิลลิลิตรต่อกิโลกรัม (ค่าพิสัยระหว่างควอร์ไทล์ 46-64 มิลลิลิตรต่อกิโลกรัม, $p < 0.001$) ผู้ป่วยในกลุ่มจำกัดความเร็วได้รับสารกระตุ้นการหดตัวกล้ามเนื้อหลอดเลือดและการช่วยหายใจด้วยเครื่องช่วยหายใจน้อยกว่า ไม่มีความต่างอย่างมีนัยสำคัญทางสถิติของการเปลี่ยนแปลงค่าซินดิแคน-1 ที่ 6 ชั่วโมงระหว่าง 2 กลุ่ม (อัตราส่วนค่าเฉลี่ยเรขาคณิตในกลุ่มจำกัดความเร็วเท่ากับ 0.82 (ค่าความเชื่อมั่น 95% เท่ากับ 0.66-1.02, $p = 0.07$) ไม่มีความต่างอย่างมีนัยสำคัญทางสถิติของเหตุการณ์ไม่พึงประสงค์ ภาวะอวัยวะล้มเหลว และการเสียชีวิตใน 90 วัน สรุป ในการกู้ชีพผู้ป่วยพิษเหตุติดเชื้อ การให้สารน้ำจำกัดความเร็วไม่สามารถลดการบาดเจ็บของชั้นไกลโคแคลิกซ์ได้อย่างมีนัยสำคัญเมื่อเทียบกับการใช้สารน้ำความเร็วมาตรฐาน

สาขาวิชา	เวชศาสตร์คลินิก	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6074853930 : MAJOR CLINICAL SCIENCES
KEYWORD: endothelial glycocalyx, sepsis, resuscitation fluid, syndecan-1, emergency department

Jutamas Saoraya : The Effects of Limited Infusion Rate of Fluid in the Early Resuscitation of Sepsis on Syndecan-1 Shedding: a Randomized Controlled Trial (LIFE3S trial). Advisor: Assoc. Prof. KHRONGWONG MUSIKATAVORN, M.D. Co-advisor: Assoc. Prof. NATTACHAI SRISAWAT, M.D.

Background: Aggressive fluid administration is recommended in the resuscitation of septic patients. However, the delivery of a rapid fluid bolus might cause harm by inducing degradation of the endothelial glycocalyx. This research aimed to examine the effects of the limited infusion rate of fluid on glycocalyx shedding as measured by syndecan-1 in patients with sepsis-induced hypoperfusion. Methods: A prospective, randomized, controlled, open-label trial was conducted between November 2018 and February 2020 in an urban academic emergency department. Patients with sepsis-induced hypoperfusion, defined as hypotension or hyperlactatemia, were randomized to receive either the standard rate (30 ml/kg/hr) or limited rate (10 ml/kg/hr) of fluid for the first 30 ml/kg fluid resuscitation. The primary outcome was the change in plasma syndecan-1 levels over six hours. Secondary outcomes included adverse events, organ failure and 90-day mortality. Results: We included 96 patients in the intention-to-treat analysis, with 48 assigned to the standard-rate strategy and 48 to the limited-rate strategy. The median fluid volume in six hours in the limited-rate group was 39 ml/kg (interquartile range [IQR] 35 – 52 ml/kg) vs. 53 ml/kg (IQR 46-64 ml/kg) in the standard-rate group ($p < 0.001$). Patients in the limited-rate group were less likely to received vasopressors (17% vs 42%; $p = 0.007$) and mechanical ventilation (20% vs 41%; $p = 0.049$) during the first six hours. There were no significantly different changes in syndecan-1 levels at six hours between the two groups (geometric mean ratio [GMR] in the limited-rate group, 0.82; 95% confidence interval [CI], 0.66 – 1.02; $p = 0.07$). There were no significant differences in adverse events or organ failure outcomes or 90-day mortality between the two groups. Conclusions: In sepsis resuscitation, the administration of resuscitative fluid with the limited-rate strategy did not significantly mitigate the glycocalyx damages compared to the standard-rate approach.

Field of Study:	Clinical Sciences	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I'm grateful for the funding from the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University, The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship and The 90th Anniversary Chulalongkorn University Fund. (Ratchadaphiseksomphot Endowment Fund).

I am also grateful to Miss Sasipha Tachaboon and Miss Janejira Dinhuzen for assisting in the laboratory methods; Miss Pimnara Peeraweranan and Stephen Kerr for helping with the statistical methods. I have learned so much from all of you.

I would like to thank everyone I got to know in the Clinical Science program, all the MSc and Ph.D. students, staff, and teachers. I enjoyed our journey together. Also, I would like to thank Professor Wasee Tulvatana for being the first person responding to my intention to study in this program and being an inspiration for me in many ways.

I truly appreciate my family for being so supportive and understanding.

Last but not least, I would like to thank Kanchana Prasith for all your help. This project could not be completed without you.

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Chapter 1 Introduction

Sepsis is a disease with high complexity and mortality rate. Even in the controlled environment in the clinical trial settings, the mortality of septic shock is still around 30%.[1-3] Early fluid resuscitation is one of the mainstay treatments of sepsis. According to the Surviving Sepsis Campaign guideline 2016 and the 2018 bundle update, 30 ml/kg of crystalloids should be given in the first 3 hours in the resuscitation of sepsis-induced hypoperfusion [4, 5].

Fluid resuscitation was usually administered in bolus fashion due to potentially early restoration of mean arterial pressure and microcirculation. However, the beneficial effects of rapid fluid bolus have been questioned. The hemodynamic effect of crystalloid bolus in the resuscitation of sepsis was minimal and short-lived [6-9]. In a clinical study, longer time to complete 30 ml/kg fluid bolus was not associated with more mortality in patients with sepsis-induced hypoperfusion [10]. Moreover, aggressive fluid therapy might lead to harm such as a higher risk of intubation in pediatric patients with septic shock [11] and increased mortality in both children [12], and adults [13] with septic shock in the resource-limited settings. In addition, an increased fluid balance was associated with increased mortality in sepsis [14].

Endothelial glycocalyx damage is one of the deleterious effects of the rapid fluid bolus. Glycocalyx has an important role in the regulation of vascular permeability. Damages to glycocalyx lead to disruption of endothelial surface layer, increase permeability and increase organ failure. Rapid fluid resuscitation was found to lead to hypervolemia and damage endothelial glycocalyx [15-17]. However, the effect of rapid fluid bolus on glycocalyx shedding in patients with sepsis has not been studied. The optimal rate of fluid resuscitation in sepsis has not been explored. It is unknown whether a limited infusion rate of fluid in the early resuscitation of sepsis could mitigate glycocalyx damages.

This study aims to compare the effects of limited versus standard infusion rate of fluid in the early resuscitation of sepsis on the syndecan-1 level, one of the biomarkers of

glycocalyx damage. Moreover, as the prognostic role of syndecan-1 in the emergency department is unclear, this study also aims to explore the association of syndecan-1 level with fluid requirements, laboratory values and clinical outcomes.

Chapter 2 Literature Review

2.1 Definition and Pathophysiology in sepsis-induced hypoperfusion and shock

According to the sepsis-3 definition, sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" [18]. Sepsis-induced hypoperfusion is defined as sepsis with hypotension or lactate ≥ 4 mmol/L at presentation [5]. Septic shock is defined as sepsis requiring vasopressor to maintain hemodynamic stability and having lactate > 2 mmol/L despite adequate fluid resuscitation [18].

Hypoperfusion in patients with sepsis is contributed by various types of shock including distributive, hypovolemic and cardiogenic shock. The distributive or septic component of septic shock results from a vasoplegic state, on both arterial and venous sides, leading to systemic hypotension and decreased venous return. Deranged microcirculation is caused by activation of a complex inflammatory cascade, resulting in diffused endothelial injury, increased capillary permeability and shunting of blood flow. Many factors contributed to the hypovolemic component, such as the inability to maintain oral intake, loss of fluid due to fever or gastrointestinal loss, third spacing of fluid and venodilatation [19]. The cardiogenic component or "septic cardiomyopathy" is a common finding in patients with sepsis. This involves diastolic dysfunction for more than half of the patients and systolic dysfunction for around ¼ of patients [20].

2.2 Fluid resuscitation in sepsis.

2.2.1 Definition of fluid bolus

Though fluid bolus therapy is one of the most common intervention in the critically ill patients especially in patients with sepsis-induced hypoperfusion, there is no standard definition of the fluid bolus. According to a worldwide survey of intensivists, wide variations in practice were discovered. Most intensivists thought that more than 500 ml of crystalloid in less than 30 minutes would be considered a fluid bolus therapy [21]. A systematic review examining previous studies describing the use of fluid bolus found that the median fluid bolus was 500 ml (range 100 to 1000 ml) given over 30 min (range

10 - 60 minutes) [9]. In the early-goal directed therapy trial, recognized as the prototype of the sepsis resuscitation, crystalloid was given as a bolus dose of 500 ml every 30 minutes without mentioning the exact rate of administration [22]. In the protocolized standard care arm of the ProCESS trial, fluid bolus was defined as 500-1000 ml in 20 minutes [2].

2.2.2 Effect of the fluid bolus in sepsis

2.2.2.1 Physiological effect

The rationale in using fluid resuscitation of sepsis-induced hypoperfusion and septic shock is due to hypovolemic component of the pathophysiology of sepsis. Fluid resuscitation can increase venous return, which in patients with fluid responsiveness, could increase stroke volume and cardiac output, as shown in the Frank-Starling curve (Figure 1). Increasing cardiac output will lead to optimize macrocirculation (e.g., blood pressure and also microcirculation of septic patients), which would lead to mitigation of organ dysfunction. However, when fluid was given more than the optimal preload, the stroke volume will not increase and would possibly lead to harm such as increasing extravascular lung water, tissue edema and paradoxically, decreased tissue blood flow [23].

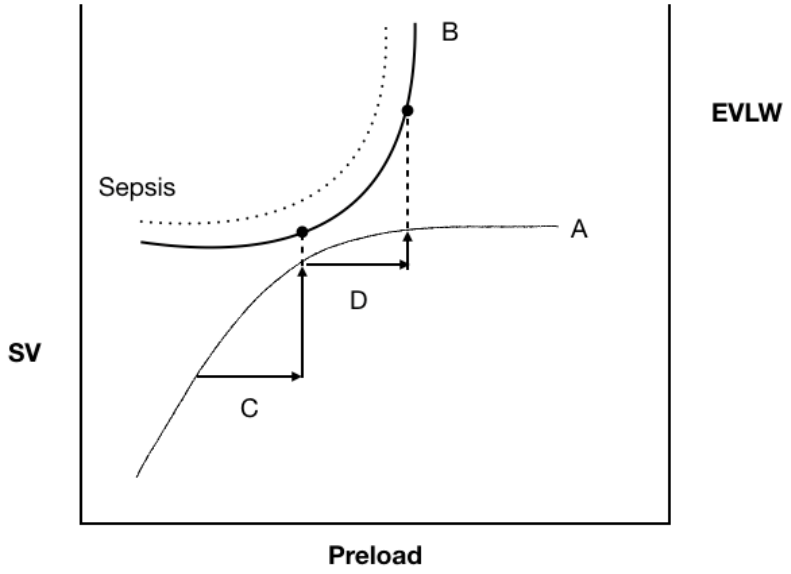


Figure 1 The Frank-Starling curve shows the effects of increasing preload on SV (A). The Marik-Phillips curve shows the effects of increasing preload on the EVLW(B). In patients with fluid responsiveness, increased preload led to increased SV and cardiac output with minimal increased EVLW (C). However, in non-responder, increased preload led to increased extravascular lung water with minimal increased SV and cardiac output (D). SV: stroke volume, EVLW: extravascular lung water (Adapted from [23].)

2.2.2.2 Hemodynamic effects of fluid bolus

The transient hemodynamic effect of the fluid bolus, especially crystalloid, has been described in both animal and clinical studies. After 20 minutes of infusion of normal saline solution (NSS) 32 ml/kg in septic rats, the plasma volume expanding effect remained only 0.6% of the infused volume [24]. Regarding the septic patients, even in the fluid responders, the cardiac index increased at 30 minutes after fluid bolus but decreased to baseline at 60 minutes [6]. A systematic review of fluid bolus therapy in sepsis found that median increases in the mean arterial pressure (MAP) were only 7.5 mmHg (range 3-11 mmHg) and 3 mmHg (range 2-7 mmHg) immediately and at 60 minutes after fluid administration, respectively [9]. In the emergency department setting, fluid bolus therapy could increase the MAP in 10 minutes, but the MAP

returned to baseline value at 1-2 hours, which occurred to ⅔ of patients with septic shock [7]. Another study of fluid bolus therapy in an emergency department also found that in 6 hours, despite aggressive fluid resuscitation, there was persistent hypotension in 40% of the patients with infection and hypoperfusion [8].

A rapid rate of fluid administration might decrease hemodynamic effect of fluid when compared to that of the slower rate. In septic guinea pigs, plasma volume expanding effect was better in the slower infusion rate in the colloid group (12ml/kg in 15 min versus in 3 hours), though not much difference was found in the crystalloid group [25]. In a volume kinetics study in human volunteers, the fraction of the infused crystalloid that remained in the plasma was higher for lower rate of infusion [26]. Another study found that the cardiac output increased for 0.02 L /min in slower fluid bolus (rate 500 ml/hr) when compared to rapid fluid bolus (rate 2000 ml/hr). However, the effect returned to baseline after infusion [27].

2.2.2.3 Clinical outcomes after rapid fluid bolus resuscitation

Early and aggressive fluid resuscitation was thought to be beneficial in septic patients. The idea of using rapid fluid bolus resuscitation came from the landmark study in 2001, the early goal-directed therapy (EGDT) trial [22], which showed that early aggressive resuscitation, including fluid therapy, resulted in improved survival in patients with septic shock. Though recent clinical trials did not show the beneficial effect on sepsis resuscitation using EGDT specifically, all alternative arms in the trials were using aggressive and rapid fluid administration in the early resuscitation. Moreover, before enrollment in the study, all patients received an average of 30 ml/kg in all treatment arms [1-3]. In a prospective, multicenter, observational study, septic patients who received a higher amount of fluid until the third day were found to have lower mortality [28]. In a retrospective cohort of single intensive care unit (ICU) studying patients with severe sepsis and septic shock, the survivors have median fluid within the first 3 hours more than non-survivors (2085 versus 1600 ml). When adjusted for the severity score, higher fluid received in the first 3 hours associated with decreased hospital mortality [29]. Currently, the surviving sepsis campaign 2016 recommends administration of fluid 30 ml/kg in the initial 3 hours based on low-quality evidence [4].

However, many studies also showed worsened outcomes in rapid fluid administration in sepsis. In septic pigs, a higher rate of fluid infusion, though resulted in better hemodynamic profiles, led to higher fluid balance and more mortality [30]. Regarding to an observational study of time to complete sepsis 3-hour bundles, a longer time to complete 30 ml/kg bolus was not associated with more mortality in patients with sepsis-induced hypoperfusion [10]. In the resource-limited settings, fluid bolus in the early resuscitation of sepsis resulted in poorer outcome. In African children with severe infection, fluid bolus increased mortality (RR = 1.44;95%CI 1.09-1.90) when compared to no fluid bolus [12]. In African adults with sepsis and hypotension, increased mortality (RR = 1.46;95%CI, 1.04-2.05) was found after implementation of early resuscitation protocol, including intravenous fluid bolus therapy [13]. Moreover, in pediatric patients with septic shock, a randomized controlled trial showed that the one who received fluid boluses of 20 ml/kg over 5-10 minutes, had higher risk of intubation than those who received boluses over 15-20 minutes [11].

Moreover, a rapid fluid bolus could lead to excessive fluid administration and positive fluid balance, which was associated with negative outcomes in patients with sepsis. Every 1-liter increase in fluid balance in the first 72 hours of sepsis was associated with an increased mortality rate of 10% [14]. Mean fluid intake and fluid balance were greater in non-survivors than in survivors in another study of septic patients in the ICU [31]. The liberal fluid strategy led to an increase in fluid balance and lengthen the duration of mechanical ventilation and intensive care in patients with acute lung injury [32].

2.3 Alternative to fluid bolus therapy: Vasopressors

As fluid bolus therapy has a transient effect on maintaining hemodynamic stability in septic patients, vasopressor, esp. norepinephrine, could instead be a more effective intervention. Physiologically, the mechanism of action of norepinephrine correlates with the pathophysiology of sepsis. In sepsis, diffuse arterial and venous dilatation leads to hemodynamic instability. Norepinephrine reverses the mechanism by constricting the vessel on both the arterial and venous sides. The arterial vasoconstriction results in increased blood pressure, while the venous vasoconstriction results in increased

stressed venous volume and increased venous return; Thus, imitating the consequence of fluid bolus without risking fluid overload [19].

Early use of vasopressor is more recognized in the resuscitation of sepsis. A retrospective study in the ICU found that early administration of norepinephrine in septic shock patients is associated with decreased mortality [33]. In a randomized controlled trial of 320 patients, comparing early use of norepinephrine after initial fluid resuscitation with placebo, the early group resulted in a higher rate of achievement of shock reversal within 6 hours with a nonsignificant trend of a lower rate of cardiogenic pulmonary edema and arrhythmia. The amount of fluid administration was about the same in both groups [34]. However, if the vasopressor was administered while decreasing fluid resuscitation, it could lead to harm [35].

Historically, vasopressor was administered in the central venous catheter due to the concern about local tissue injury. However, vasopressors could be safely administered via the peripheral route. Extravasation and local tissue injury after peripherally-administered norepinephrine mostly occurred in prolonged infusion, which results in minor injuries such as skin blanching and edema [36].

2.4 Endothelial glycocalyx

The endothelial glycocalyx is a polysaccharide-rich layer consisting of membrane-bound glycoproteins and proteoglycans that extends from the endothelium. Glycocalyx, in coupled with blood-borne proteins especially albumins, cations and water, forms the endothelial surface layer [37] (Figure 2).

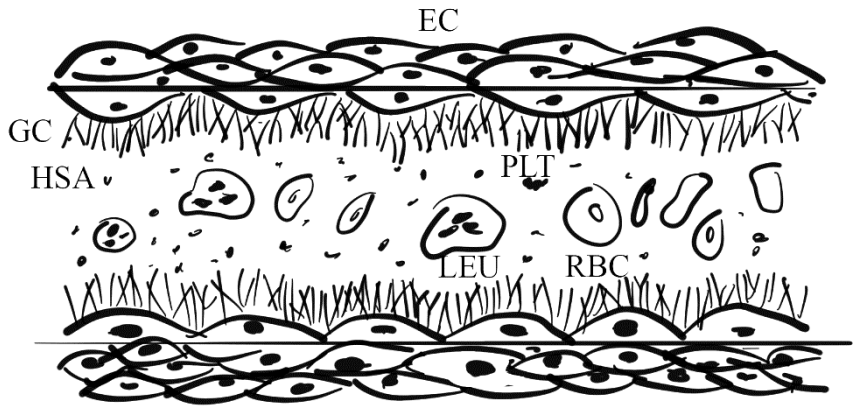


Figure 2 Healthy capillary with intact layer of endothelial glycocalyx. EC: endothelial cells, GC: glycocalyx, RBC: red blood cells, PLT: platelets, LEU: leukocytes, HSA: human serum albumin. (Adapted from [38])

Functions of the endothelial glycocalyx and the endothelial surface layer include working as a barrier to molecules larger than albumin (> 70 kDa), regulating vascular permeability, influencing blood cell-endothelium interactions and controlling microenvironment, which are adhesion molecules, coagulation, fibrinolytic and hemostatic system [39].

Destruction of the endothelial glycocalyx could be caused by many factors, including ischemia[40], inflammation [41], hypervolemia[17], hyperglycemia [42], and hypoxia [43]. Destruction of the glycocalyx leads to increased capillary permeability [44]. Many treatment modalities have been shown to protect or restore the endothelial glycocalyx, including N-acetyl cysteine [42] and hydrocortisone [45, 46] (Figure 3).

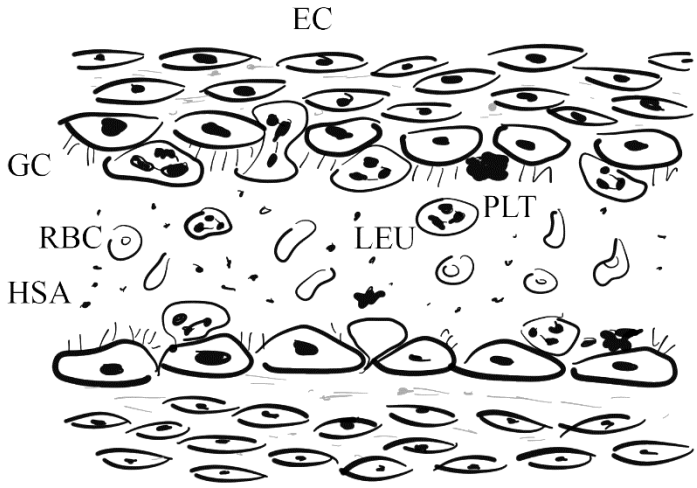


Figure 3 Damaged capillary with damaged endothelial glycocalyx, disrupted endothelial barrier with leakage of plasma and interstitial edema. EC: endothelial cells, GC: glycocalyx, RBC: red blood cells, PLT: platelets, LEU: leukocytes, HSA: human serum albumin. (Adapted from [38])

The glycocalyx can be measured *in vivo* by 2 broad categories: laboratory methods and direct imaging. The laboratory method is to measure components of endothelial glycocalyx e.g., syndecan-1 (CD138), hyaluronan, or heparan sulfate in the plasma as a marker of glycocalyx shedding and damages. The imaging method is to use intravital microscopy or electron microscopy to direct visualize endothelial glycocalyx. The degree of glycocalyx damages quantified as Perfused Boundary Region (PBR) can be measured in sublingual microvasculature using orthogonal polarization spectral (OPS), sidestream dark field (SDF), or incident dark-field imaging technique [38, 47].

However, in the clinical setting, the laboratory method is more practical to conduct. One of the components of glycocalyx that are largely studied is syndecan-1. It is an indirect marker of glycocalyx degradation. Changes in plasma levels of syndecan-1 were negatively correlated with changes in glycocalyx thickness and positively correlated with changes in microvascular permeability [44]. The level of syndecan-1 also has a clinical correlation in patients with sepsis. Higher-level was associated with risks of intubation after large-volume fluid resuscitation [48] and organ failures [49].

The level could predict the patients developing disseminated intravascular coagulation (DIC) [50] and differentiated non-survivors from survivors [51].

2.5 Administration of fluid and glycocalyx shedding

Several studies exhibit the link between hypervolemia, especially from rapid fluid administration and glycocalyx shedding. Resuscitation of hemorrhagic rats using lactated ringer's solution (LRS) resulted in increased plasma syndecan-1 up to 5 times of the baseline and reduced glycocalyx thickness up to 20 % of the baseline level [44]. The shedding of syndecan-1 was more pronounced when using normal saline administration compared with LRS [52]. In septic rabbits, more rapid fluid administration (30 ml/kg/h) resulted in more syndecan-1 shedding when compared to a slower rate of fluid administration (10 ml/kg/hr) [53]. In humans, the infusion of ringer's acetate solution 1000 ml in 40 minutes increased plasma hyaluronan at the end of the infusion [15]. The increased syndecan-1 level was observed after the bolus of LRS 750 ml over 15 min [16]. Volume loading with colloid (20ml/kg) induced release of syndecan-1 and hyaluronan by 80% when compared to acute normovolemic hemodilution [17]. The phenomenon was explained by the fact that volume loading increases cardiac filling pressure and increases the release of natriuretic peptides[17]. The natriuretic peptides shed the glycocalyx components especially syndecan-1 and results in glycocalyx damages. [54, 55].

However, the effect of rapid fluid bolus on glycocalyx shedding in patients with sepsis has not been studied. The optimal rate of fluid resuscitation in sepsis has not been explored. It is unknown whether the limited infusion rate of fluid in the early resuscitation of sepsis could lead to mitigation of glycocalyx damages.

Chapter 3 Methods

3.1 Objectives

3.1.1 Primary objective

To compare the effects of limited versus standard infusion rate of fluid in the early resuscitation of sepsis on syndecan-1 levels at 6 hours.

3.1.2 Secondary objectives

1. To compare amount fluid input at 6, 24, and 72 hours after randomization when using limited versus standard infusion rate in the early resuscitation of sepsis.

2. To compare lactate clearance at 6 hours when using limited versus standard infusion rate of fluid in the early resuscitation of sepsis

3. To compare effects of limited versus standard infusion rate of fluid in the early resuscitation of sepsis on clinical outcomes including PaO2/FiO2 (P/F) ratio, mortality, hospital length-of-stay, organ-failure free days.

4. To explore the correlation of syndecan-1 at baseline and 6 hours after the resuscitation period with fluid requirement and clinical outcomes in ED patients with sepsis.

5. To explore the association between syndecan-1 and other laboratory values, including N-terminal pro-b-type natriuretic peptide (NT-proBNP) level.

3.2 Research questions

3.2.1 Primary research question

In patients with sepsis-induced hypoperfusion, does resuscitation with a limited infusion rate of fluid result in less syndecan-1 level at 6 hours than resuscitation with the standard rate?

3.2.2 Primary research hypothesis

In patients with sepsis-induced hypoperfusion, resuscitation with a limited infusion rate of fluid results in less syndecan-1 level at 6 hours than resuscitation with the standard rate.

3.2.3 Secondary research questions

1. In patients with sepsis-induced hypoperfusion, does resuscitation with a limited infusion rate of fluid result in lower fluid input at 6, 24 and 72 hours than resuscitation with the standard rate?

2. In patients with sepsis-induced hypoperfusion, does resuscitation with a limited infusion rate of fluid result in higher lactate clearance at 6 hours than resuscitation with the standard rate?

3. In patients with sepsis-induced hypoperfusion, does resuscitation with limited infusion rate of fluid result in better clinical outcomes (e.g., increase P/F ratio, decreased mortality, decreased hospital length-of-stay, and increased organ-failure free days) than resuscitation with standard rate?

4. In patients with sepsis-induced hypoperfusion in the emergency department, are there any correlations between the syndecan-1 levels at baseline and 6 hours with fluid requirement and clinical outcomes?

5. In patients with sepsis-induced hypoperfusion in the emergency department, are there any correlations between the syndecan-1 levels at baseline and 6 hours with laboratory values?

3.2.4 Secondary research hypotheses

1. In patients with sepsis-induced hypoperfusion, resuscitation with a limited infusion rate of fluid results in lower fluid input at 6, 24, and 72 hours than resuscitation with the standard rate.

2. In patients with sepsis-induced hypoperfusion, resuscitation with a limited infusion rate of fluid results in higher lactate clearance at 6 hours than resuscitation with the standard rate.

3. In patients with sepsis-induced hypoperfusion, resuscitation with a limited infusion rate of fluid results in better clinical outcome (e.g., increase P/F ratio, decreased mortality, decreased hospital length-of-stay, and increased organ-failure free days) than resuscitation with the standard rate.

4. In patients with sepsis-induced hypoperfusion in the emergency department, the degree of glycocalyx shedding, as measured by syndecan-1, is associated with the fluid requirement, worsening clinical outcomes, and mortality.

5. In patients with sepsis-induced hypoperfusion in the emergency department, the degree of glycocalyx shedding, as measured by syndecan-1, is associated with worsening laboratory values.

3.3 Keywords

Sepsis, endothelial glycocalyx, syndecan-1, emergency department, resuscitation fluid

3.4 Conceptual framework

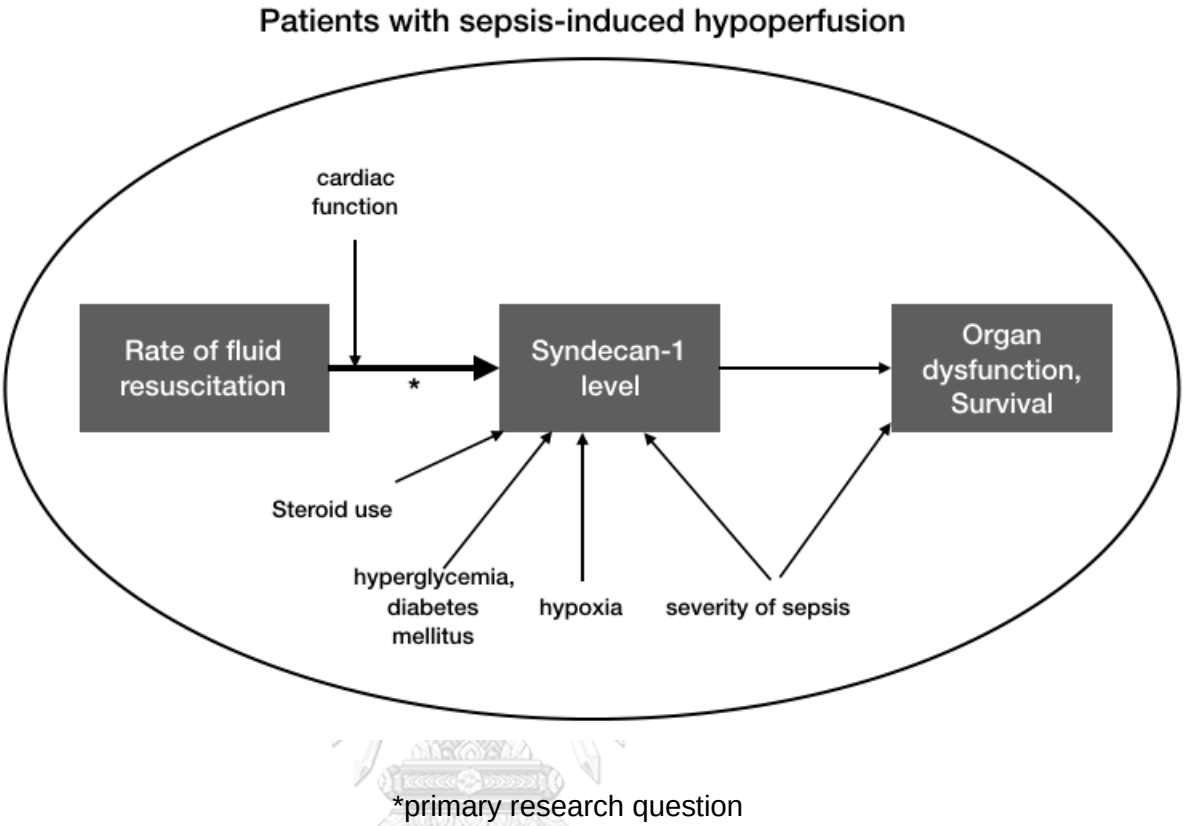


Figure 4 Conceptual framework of this study

3.5 Study definitions

1. Sepsis: according to the sepsis-3 definition, sepsis is defined as “life-threatening organ dysfunction caused by a dysregulated host response to infection” [18].

2. Sepsis-induced hypoperfusion: the sepsis with hypotension or lactate ≥ 4 mmol/L [5].

3. Fluid intake: volumes of fluid administered to the participant, including intravenous fluid for the purpose of resuscitation, the mixture of medicine or maintenance, and enteral fluid.

4. Fluid output: sum of the volumes of urine output, ultrafiltration fluid, fluid from drain and estimated gastrointestinal losses.

5. Fluid balance: subtraction of fluid output from the fluid intake.

6. Lactate clearance in 6 hours: calculated by subtracting the lactate level at 6 hours from the initial lactate level and divided by the initial lactate level (i.e., [(Initial lactate – lactate at hour 6)/Initial lactate] ×100%).

7. Shock reversal: defined as patients who have both mean arterial pressure (MAP) ≥ 65 mmHg and lactate clearance > 10% in 6 hours.

8. P/F ratio: PaO2/FiO2 ratio. The PaO2 is measured by arterial blood gas. The FiO2 is as per the setting of the ventilator or per the estimation of oxygen supplement.

9. Organ failure-free day to day 28: days alive and free from mechanical ventilation, renal replacement therapy or vasopressors until 28 days. If the patient dies prior to day 28, the organ failure-free day will be 0.

10. Vasopressor-free day to day 28: days alive and free from vasopressors until 28 days. If the patient achieves vasopressor cessation, return to receiving vasopressor and achieved vasopressor cessation again, vasopressor-free day will be counted based on the time of the final cessation of vasopressor prior to day 28. If the patient dies prior to day 28, the vasopressor-free day will be 0.

11. Ventilator-free day to day 28: days alive and free from a ventilator or assisted breathing until 28 days. If the patient achieves unassisted breathing, return to receive assisted breathing and achieved unassisted breathing again, ventilator-free day will be counted based on the time of the final cessation of ventilator prior to day 28. If the patient dies prior to day 28, the ventilator-free day will be 0.

12. Renal replacement therapy-free days to day 28: days alive and free from renal replacement therapy (RRT) until 28 days. If the patient achieves a cessation of

RRT, return to receive RRT and achieve the cessation of RRT again, RRT-free day will be counted based on the time of the final cessation of RRT prior to day 28. If the patient dies prior to day 28, the RRT-free day will be 0.

13. The limited rate of fluid resuscitation group is defined as resuscitation using lactated Ringer's solution (LRS) infusion at the rate of 10 ml/kg/hr (via infusion pump) for the first 30 ml/kg of fluid resuscitation. After completion of the designated fluid, further fluid resuscitation will be given according to the physician's discretion, but at the rate of not more than 10 ml/kg/hr until 6 hours.

14. The standard rate of fluid resuscitation group is defined as resuscitation using lactated Ringer's solution (LRS) infusion at the rate of 30 ml/kg/hr (via infusion pump, with the maximum rate of 2000 ml/hr) for the first 30 ml/kg of fluid resuscitation. After completion of the designated fluid, further fluid resuscitation will be given according to the physician's discretion, but at the rate of not more than 30 ml/kg/hr (or maximum rate of 2000 ml/hr) until 6 hours.

3.6 Research design

This is a single-center, with equal randomization (1:1), open-label, investigator-initiated, parallel-group study that was conducted in the emergency department of King Chulalongkorn Memorial Hospital. The emergency department has the census of 40,000 visits per year, and stands in a 1,400-bed tertiary care center.

3.7 Research Methodology

3.7.1 Population

All patients aged 18 or over, presenting to the emergency department with suspected sepsis were screened in the triage area of the emergency department, and the investigators will be notified.

3.7.1.1 Target population

Adults aged 18 or over, presenting to the emergency department with sepsis-induced hypoperfusion, which was defined as sepsis with hypotension or lactate ≥ 4 mmol/L.

3.7.1.2 Inclusion criteria

1. adults aged 18 or over
2. with suspected sepsis according to the sepsis-3 definition identified by [18]
    a. suspected infection with qSOFA ≥ 2/3
        i. alteration in mental status
        ii. Systolic Blood Pressure (SBP) ≤ 100 mmHg
        iii. Respiratory rate (RR) ≥ 22/min
3. and hypoperfusion as by clinician's decision to use fluid bolus therapy for 30 ml/kg including
        i. Systolic Blood Pressure (SBP) < 90 mmHg,
        ii. MAP < 65 mmHg
        iii. lactate ≥ 4 mmol/L.

3.7.1.3 Exclusion criteria

1. Received resuscitation fluid for more than 500 ml.
2. Severe hypotension (SBP < 70 mmHg).
3. Suspected other main causes of hypoperfusion (obstructive, cardiogenic, hypovolemic such as gastrointestinal hemorrhage).
4. Concurrent acute heart failure or known left ventricular ejection fraction (LVEF) less than 40% or poor LVEF by eyeballing on point of care ultrasound (POCUS).
5. End stage renal disease (ESRD) with chronic RRT.
6. Suspected infection from microorganisms other than bacteria.

7. Potentially need for immediate surgery in 6 hours.
8. Body mass index (BMI) ≥ 30 kg/m^2.
9. Concurrent acute traumatic brain injury.
10. Do-Not-Attempt-Resuscitation (DNAR) order status.
11. Transferred from another hospital.
12. Pregnancy.

3.7.2 Informed consent process

The investigators or the research assistants, who are not actively involved in the treating team of the patients would explain the research objectives and procedures to the eligible patients, or the legal representatives if the patients lack decision-making capacity. The information sheet explaining the details of the study was given. They could freely ask the questions and the explanation and answers would be provided. If they volunteer to participate in the study, the written informed consent would be obtained. The informed consent would be obtained from the legal representatives during the first 500 ml of the administered research fluid and would be acquired from the patients once they can provide one. They could withdraw consent any time and the decision would not affect the standard treatment of patients. All patients, whether participating or not, or withdrawn from the study, was treated according to the current standard treatment of sepsis.

3.7.3 Randomization and procedures

Participants was randomized into 2 groups in a 1:1 ratio using block randomization, with blocks of varying size of 4,6 and 8. The randomization sequence was created using a random number generator. The allocations were concealed in the opaque, sealed envelopes. The details of the procedure of each group are:

1. The intervention group: the limited rate of fluid resuscitation is defined as resuscitation using lactated Ringer's solution (LRS) infusion in the rate of

10ml/kg/hr (via infusion pump) for the first 30 ml/kg of fluid resuscitation. After completion of the designated fluid, further fluid resuscitation will be given according to the physician's discretion, but at the rate of not more than 10 ml/kg/hr until 6 hours.

2. The control group: the standard rate of fluid resuscitation is defined as resuscitation using lactated Ringer's solution (LRS) infusion in the rate of 30 ml/kg/hr (via infusion pump, with maximum rate of 2000 ml/hr) for the first 30 ml/kg of fluid resuscitation. After completion of the designated fluid, further fluid resuscitation will be given according to the physician's discretion, but at the rate of not more than 30 ml/kg/hr (or maximum rate of 2000 ml/hr) until 6 hours.

All patients received the standard treatment for sepsis, including early appropriate antibiotics administration, source control and the MAP target of 65 mmHg.

The MAP was monitored every 5 minutes using non-invasive blood pressure monitoring until achieving hemodynamic stability (defined as MAP >65 mmHg for at least 3 consecutive measurements). If the hemodynamic stability could not be achieved within 15 minutes, norepinephrine would be peripherally administered in the concentration of 4 mg diluted in 250 ml at the rate of 5 ml/hr (starting dose = 1.3 mcg/min) and titrated to keep MAP > 65 mmHg. After achieving hemodynamic stability, the blood pressure would be monitored every 15 minutes for 1 hour and then every 1 hour until 6 hours. The decision to insert a central venous catheter, arterial catheter or to use corticosteroids would depend on the clinician's judgment.

3.7.4 Safety limit for early protocol termination

- During the first 30 ml/kg of fluid resuscitation, the protocol could be terminated if the participants exhibited signs of fluid overload, including crepitation of lungs, SpO2 decrease > 3%, or respiratory rate (RR) increase > 5 /min

- During the entire 6 hours, the protocol could be terminated if the participants encountered refractory hypotension despite optimizing vasopressors, and the clinician would like to administration fluid faster than the designated rate.

- The protocol could be terminated upon the physician's decision as per the patients' benefits and non-maleficence.

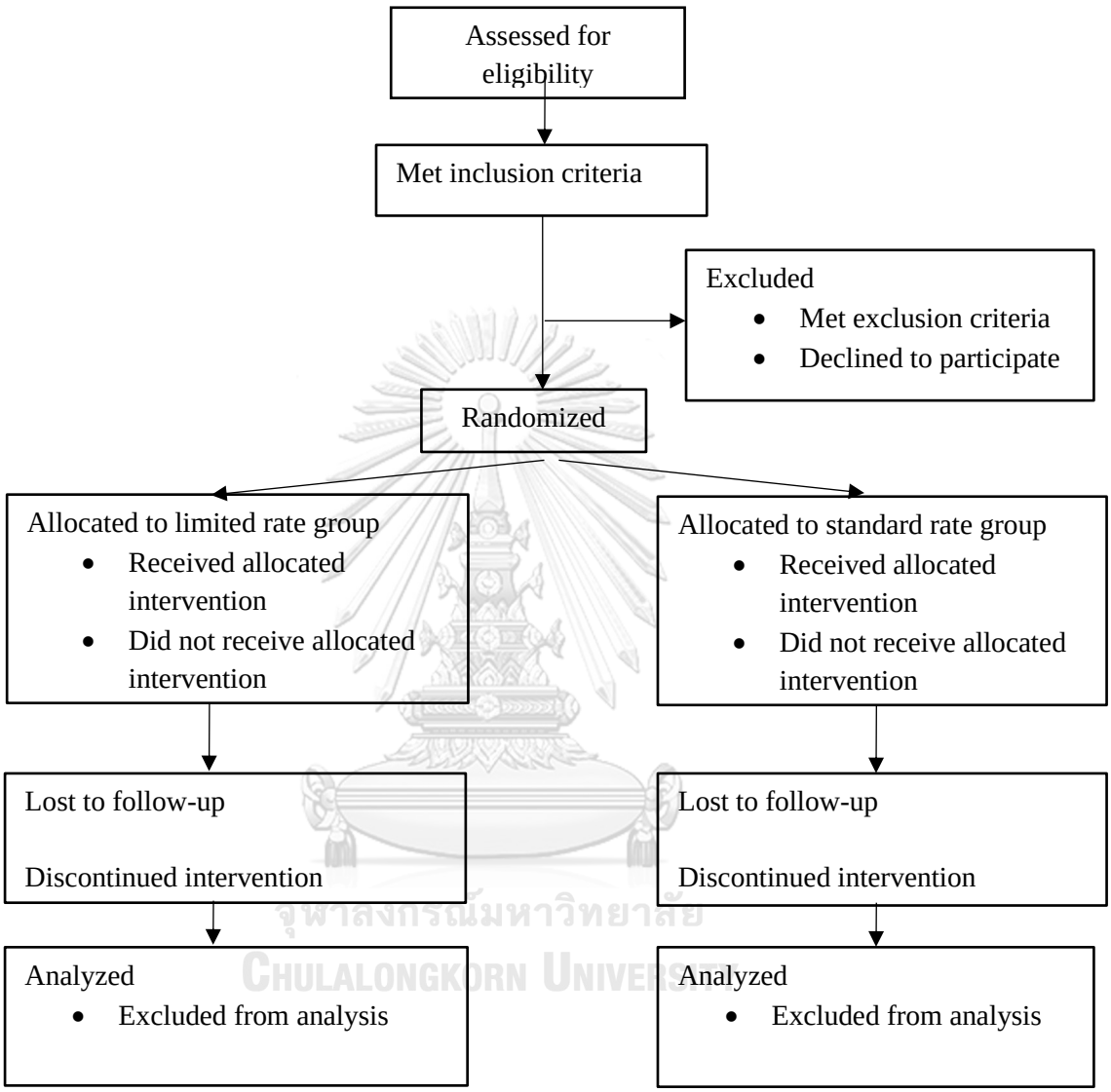
3.7.5 Rescue therapy

- If the clinician assessed that the patient developed fluid overload, when appropriate, the diuretics (e.g., furosemide) could be prescribed to enhance elimination of fluid.

- If the clinician assessed that the patient needed fluid faster than the designated rate, fluid can be administered faster as per clinician' discretion

- In an event of norepinephrine extravasation [36]
    - Stop the infusion
    - Aspirate the drug as much as possible
    - Infiltrate terbutaline 1 mg diluted in 10 ml of normal saline
        - 5 ml inject through catheter
        - 5 ml into the affected area
    - Remove catheter

Figure 5 CONSORT Flow Diagram



3.7.6 Blood sampling

Blood sample was collected at enrollment determine baseline syndecan-1, lactate, PaO2 and NTproBNP. Another blood sampling was conducted at 6 hours to send for syndecan-1 level, lactate and PaO2. NT-proBNP levels were measured using electrochemiluminescence immunoassay analysis (Roche Diagnostics, Mannheim, Germany).

To measure the syndecan-1 level, 5 ml of whole blood was collected into the EDTA tubes and kept in the refrigerator before centrifugation and stored at -80 C. The enzyme-linked immunosorbent assay (ELISA) (Abcam, Cambridge, MA) was used to measure syndecan-1 level. The protocol for the ELISA was provided in the kit instruction and could be summarized as in the followings:

1. Removal appropriate number of antibody coated well strips
2. Equilibrating all reagents to room temperature (18 – 25 degree Celsius)
3. Preparing all the reagents, samples and standards including diluting the standard diluent buffer, wash buffer and control solution
4. Adding 100 µL of each standard including blank controls to the appropriate well
5. Add 100 µL of sample and 1x Control solution to the appropriate well
6. Adding 50 µL of prepared Biotinylated anti-syndecan-1.
7. Closing the cover and incubating at room temperature for one hour.
8. Removing the cover and washing the plate as follows:

    8.1 Aspirating the liquid from each well

    8.2 Adding 300 µL of 1x Wash Buffer into each well

    8.3 Aspirating the liquid from each well.

    8.4 Repeat for a total of three washes

9. Adding 100 µL of Streptavidin-HRP mix to each well. Re-covering and incubating at room temperature for 30 minutes.

10. Washing as in the step 8.

11. Aspirating and washing each well. Adding 100 µL of TMB solution to each well and incubate in the dark for 12-15 minutes at room temperature

12. Adding 100 µL of Stop Reagent into each well.

13. Reading absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength.

The remaining plasma would be kept at -80 C for 1 year, for further analysis if needed.

3.7.7 Sample size calculation

We would like to detect a reduction of syndecan-1 of 81 ng/ml (SD 109 ng/ml) according to the study of Steppan et al [41] with a two-sided 5% significance level and a power of 90%. Using the formula below

$$n\ per\ group\ = \frac{2(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2\sigma^2}{MCD^2}$$

$Z_{1-\frac{\alpha}{2}}$ = 1.96

$Z_{1-\beta}$ = 1.28

σ = 109 ng/ml

Minimal clinical difference (MCD) = 81 ng/ml

$$n\ per\ group\ = \frac{2(1.96 + 1.28)^2 109^2}{81^2}$$

$$n\ per\ group\ = 38.25\ \approx 39$$

When given dropout rate of 10%, adjusting the n in the group according to the formula:

$$adjusted\ n\ = \frac{n}{(1-d)}$$

when d = dropout of 20 % = 0.2

$$adjusted\ n\ = \frac{n}{(1-0.2)}$$

$$adjusted\ n\ = \frac{39}{0.8}$$

$$adjusted\ n\ = 48.75\ \approx 49$$

$$Total\ \ n\ = 49\ \times 2 = 98\ patients$$

We anticipated 12-month recruitment period to allow enrollment of this number of patients.

3.7.8 Data collection

The data was recorded in the case record form as in detail below:

At baseline, we collected the baseline characteristics (e.g., age, sex, underlying diseases), vital signs, laboratory tests, including blood culture, lactate level and syndecan-1 level. The baseline LVEF estimated by POCUS, and the baseline Plethysmograph variability index (PVI) obtained by a pulse oximeter probe were recorded.

During intervention in hour 0-1, the vital signs during intervention was collected.

During hour 0-6, the patients were monitored for the termination rules and the adverse effects during the intervention. If the patients were admitted to the hospital before 6 hour-period, the research nurses/ investigators would continue monitoring the research

procedure and were responsible for blood collection at hour 6. The admitting ward was informed in advance about this research project and its protocol.

At hour 6, we collected the syndecan-1 level, lactate level, vital signs, P/F ratio, signs of organ failure, and treatment that was administered to the patients, including vasopressor, corticosteroid, antibiotics, total fluid volume, and the use of mechanical ventilation.

At hour 24 and 72, we collected the fluid input, fluid output, and fluid balance.

At day 28, we collected the organ failure-free days until day 28, mortality rate, and microbiological investigation data.

At day 90, we collected the final outcomes, including hospital length of stay, hospital free day until day 90, and mortality at day 90 by using telephone follow-up and associated medical records.

Table 1 Timeline of the study protocol

			study period								
	Enrollment	Allocation	Post allocation (hourly)					Follow up			
Timeline	-t1	T0	T1	T2	T3	T4	T6	T24	T72	day 28	day 90
Enrollment											
Eligibility screen	X										
Informed consent	X										
Randomization		X									
Intervention											
Limited rate			----------				----------				
Standard rate			----------				----------				
Research blood sampling		x					x				
Assessment											
Baseline data		x									
BP & MAP		x	x				x				
Fluid input							x	x	x		
Fluid balance								x	x		
Organ failure free day										x	
Hospital free day											x
Mortality										x	x

3.7.9 Outcome Measurement

3.7.9.1 Primary outcome

Change of syndecan-1 at hour 6

3.7.9.2 Secondary outcome:

Early physiologic parameters

- Lactate clearance in 6 hours
- Percentage of patients with MAP ≥ 65 mmHg at hour 1 and hour 6
- P/F ratio at hour 6
- Percentage of patients with shock reversal

Adverse event

- Cardiogenic pulmonary edema
- New arrhythmia
- Extravasation of norepinephrine
- Early termination of the study protocol and the reasons

Clinical outcomes

- Hospital length of stay
- Fluid input at hour 6
- Fluid input and balance in 24 and 72 hours

Organ-failure outcome

- Organ failure-free day to day 28
- Requirement for intubation, ventilator-free days to day 28
- Requirement for new renal replacement therapy (RRT), RRT-free days to day 28
- Requirement for vasopressor, duration of vasopressors, vasopressor-free days to day 28

Mortality

- All-cause mortality at day 28 and day 90
- Time to death

3.7.10 Data analysis and statistics

Data were presented in mean (SD) or median (IQR) depending on the distribution. Due to highly skewed data, syndecan-1 levels were log-transformed to generate normal distributions and are reported as geometric means with 95% confidence intervals. Categorical data are reported as proportions. The primary outcome (the differences in change of syndecan-1 level at 6 hours between groups) was analyzed using linear regression and is reported as a geometric mean ratio (GMR). The analysis was conducted on an intention-to-treat analysis and the per-protocol-analysis. The per-protocol analysis will censor participants once they have ceased their randomized treatment. If important prognostic characteristics at baseline were unbalanced between the randomized arms, adjusted analysis using multiple linear regression would be conducted. Secondary outcomes were compared using multiple linear regression, independent t-test, paired t-test, Chi-square test or Fisher's exact test or time to event methods, depending on types of data. We did not impute missing data. However, the numbers of observations in the analysis are reported. Statistical significant level was

determined at $p < 0.05$. All analyses were performed using STATA version 16 (College Station, TX, USA).

Secondary analysis assessed the primary outcome in the pre-specified subgroup for the purpose of hypothesis generation.

Pre-specified Subgroup analysis included

- Syndecan-1 level at baseline: high vs low (according to the median level)
- APACHE II score: 0-19 vs >19
- baseline NTproBNP level: ≤ 900 pg/ml vs > 900 pg/ml
- lactate level: <4 vs ≥ 4 mmol/L
- PVI: <13 VS ≥ 13%

The post-hoc analysis was conducted to explore the differences in clinical outcomes of the patients who received early vasopressors and those who did not. Early vasopressor was defined as initiating the vasopressor to maintain hemodynamic stability within the first hour after randomization. The outcomes of the two groups were compared using independent t-tests, Chi-square test or Wilcoxon-rank sum test as appropriate.

The post-hoc analysis explored the correlation of syndecan-1 at baseline and 6 hours after the resuscitation period with fluid requirements, laboratory values and clinical outcomes in ED patients with sepsis. The patients with complete baseline syndecan-1 data were included. Categorical variables are compared by the Chi-square test. Continuous variable distributions were assessed by visually inspecting histograms, and formal comparisons were made between baseline characteristics using independent t-tests or Wilcoxon-rank sum test as appropriate. A Wilcoxon signed-rank test was used to assess the change of syndecan-1 level from baseline (T0) to 6 hours (T6). Correlations between biomarkers and characteristics, laboratory values, and outcomes were analyzed using Spearman's ρ. The difference of syndecan-1 level with different clinical outcomes was compared between groups using the Wilcoxon-rank sum test. To

determine the association between 90-day mortality and syndecan-1 level, the syndecan-1 was stratified into quartile, and the risk of mortality was compared between quartiles using the Chi-square test. Syndecan-1 levels were dichotomized into high and low levels according to the optimal cutoff as identified by Youden's index. Univariable logistic regression analysis was conducted to identify the clinical factors that were associated with 90-day mortality. The variables with a p-value < 0.1 were initially included into the multivariable logistic regression model and backward stepwise elimination was conducted to maintaining variables with a $p < 0.05$ in the models. The discriminative abilities of the models were assessed using the area under the receiver operating characteristic curves (AUROC). We did not impute the missing values, but we reported the number of observations used for calculation. Analyses were performed by STATA version 16 (College Station, TX, USA) and Prism 8 (GraphPad Software, CA, USA). Statistical significance was defined as a two-sided $p < 0.05$.

3.8 Ethical considerations

3.8.1 Respect for person

Prior to enrollment, the detailed information was given verbally and in the information sheet. The patients or their legal representative could freely ask the questions and the explanation and answers would be provided. If they volunteer to participate in the study, the written informed consent would be obtained. The participation in the study is voluntary and they could withdrew consent any time and the decision will not affect the standard treatment of patients. All patients, whether participating or not, or withdrawn from the study, were treated according to the current standard treatment of sepsis.

Confidentiality: the case record form of each patient did not contain the patient's identifiers (e.g., hospital number or identification number). The case record form and the signed informed consent were kept in two separated locked cabinets with access limited to the study investigators involving in data collection and entry.

3.8.2 Beneficence/ Non-maleficence

Potential benefits to the participants and others: The participants both in the intervention and control group might have a direct benefit regarding to the close monitoring.

Risks to the subjects: Both intervention and control group were similar that administration of at least 30 ml/kg is according to the current guideline in the treatment of sepsis-induced hypoperfusion. The difference in the rate of infusion between group, which was not defined specifically in the guideline, could somehow be encountered in everyday practice. Moreover, peripherally administered norepinephrine is a usual practice in the study setting. Therefore, we do not expect this to pose an increased risk to the patients when compared to the usual risks of resuscitation in sepsis, including pulmonary edema, persistent hypotension, or extravasation of epinephrine. However, harm will be limited using strict exclusion criteria that exclude patients with severe hypotension or frank heart failure and decreased LVEF.

Protection against risks: The protocol incorporated close monitoring for adverse events. The termination of the protocol and the rescue therapy were stated and the physician could stop the protocol any time that they think the harm can occur from the intervention.

3.8.3 Justice

The study had strict inclusion and exclusion criteria. The randomization process was robust by using vary size of block randomization process and presence of allocation concealment. Moreover, all patients were treated according to the study protocol with close monitoring.

3.9 Expected or anticipated benefit gain

The investigators wish to gain more understanding of the early resuscitation of sepsis including association of the rate of infusion and the glycocalyx shedding, and the clinical outcome. The result of this study could lead to further larger trial and probably change the practice of early resuscitation of sepsis.

3.10 Challenges

1. May encounter slow enrollment rate

2. May encounter missing primary outcome data (syndecan-1 level)

Solution: Periodically announce the study information to the emergency medicine residents and nurses, especially the triage nurses to enhance enrollment rate. The relevant providers will be trained to ensure adhering to the study protocol.

3.11 Risk and investigator's responsibility

1. The patients' blood will be drawn for the additional 10 ml for the analysis of the syndecan-1 level.

2. The patients may encounter the usual risks of the resuscitation of sepsis.

The investigator will ensure that blood collection process for the analysis of the study will be incorporated into the mandated blood sampling if possible, in order to mitigating pain associated with additional blood sampling in this study.

The protocol incorporates close monitoring for adverse events. The termination of the protocol and the rescue therapy are stated and the physician can decide to stop the protocol any time that they think the harm can occur from the intervention.

3.12 Timeline and tabulation of research activities

The total research timeline was 2 year and 6 months, as shown in Table 2.

Table 2 Research Timeline

Process	2018				2019				2020	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Proposal preparation	x									
Proposal examination		x								
IRB approval		x	x							
Recruitment				x	x	x	x			
Data analysis								x		
Manuscript preparation								x		
Thesis examination									x	
Abstract presentation										x
Submit for publication										x

3.13 Venue of the study

The study was conducted at the emergency department, King Chulalongkorn Memorial Hospital. The laboratory test of syndecan-1 level was analyzed at the Excellent Center for Critical Care Nephrology, Division of Nephrology, Department of Medicine, Chulalongkorn University

3.14 Approval from the institutional review board.

This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB No. 431/61) and was registered with the Thai Clinical Trials Registry (TCTR20181010001). This trial is reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Chapter 4 Results

4.1 Participants

From November 2018 to February 2020, 249 patients were screened for eligibility, with 146 patients met exclusion criteria, and five patients refused to participate in the trial. A total of 98 patients were randomized to either the standard rate or the limited rate group. One patient in each group was excluded from the analysis because they met the exclusion criteria of undergoing emergency surgery. Regarding the primary outcome, the syndecan-1 results were missing in two cases of the standard rate groups and four cases of the limited infusion rate groups due to administrative reasons and loss of follow-up. In summary, a total of 46 and 44 participants were analyzed for the primary outcome in the standard and limited infusion rate group, respectively. A total of 48 patients per group were analyzed regarding all other analyses not related to the syndecan-1 test. The patient flow diagram was shown in Figure 6. The baseline characteristics of patients in both groups are comparable, but the patients in the limited rate group were generally of greater hemodynamic stability and had higher prevalence of previous systemic steroid use (Table 3).

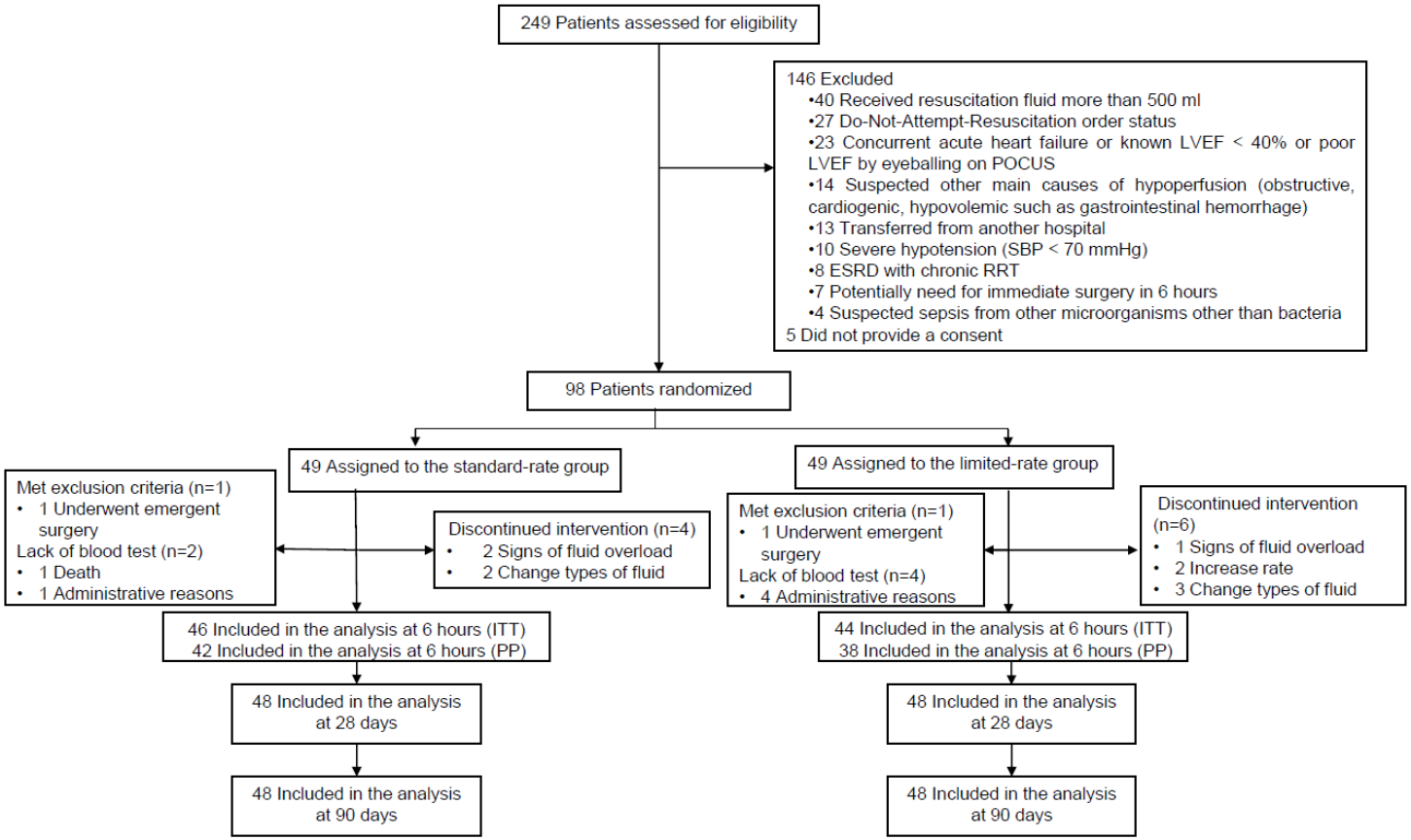


Figure 6 Flow diagram of enrollment, intervention allocation, follow-up, and data analysis. LVEF: left ventricular ejection fraction; POCUS: point-of-care-ultrasound; SBP: systolic blood pressure; ESRD: end-stage renal disease; RRT: renal replacement therapy; ITT: intention-to-treat; PP: per protocol

Table 3 Baseline Characteristics

	Standard rate (n = 48)	Limited rate (n = 48)
Age (years)	72 (16)	70(18)
Sex (female)	18 (38%)	19(40%)
Body weight (kg)	49.3 (7.9)	54.8 (11.8)
Charlson comorbidity index	5 (4, 6.5)	5 (3, 7)
Comorbidities		
Cerebrovascular disease	30 (63%)	23 (48%)
Diabetes mellitus	21 (44%)	20 (42%)
Malignancy	15 (31%)	18 (38%)
Ischemic heart disease	3 (6%)	8 (17%)
Chronic kidney disease	1 (2%)	5 (10%)
Hypoperfusion defined by:		
Lactate ≥ 2 mmol/L	44 (89.8%)	42 (85.7%)
Hemodynamic instability	23 (48%)	13 (27%)
Systolic blood pressure (mmHg)	105.9 (32.8)	114.3 (29.8)
Diastolic blood pressure (mmHg)	58.4 (20.2)	66.5 (17.7)
Mean arterial pressure (mmHg)	74.6 (22.7)	82.3 (19.9)
Body temperature (degree Celsius)	38.5 (1.3)	38.1 (1.2)

Heart rate (/min)	117.8 (28.1)	119.6 (24.3)
Respiratory rate (/min)	24.3 (7.1)	24.0 (6.3)
Ambient air pulse oximetry (%)	90.2 (12.0)	93.3 (7.7)
Currently use systemic steroid	5 (10%)	12 (25%)
APACHE II	18.0 (13.0, 24.5)	15.5 (11.0, 20.0)
SOFA	5(2,6)	4(2,5)
Good LVEF	44 (92%)	41 (85%)
Lactate (mmol/L)	4.9(3.2)	4.4(2.4)
Baseline NT-proBNP (pg/ml)*	950.7 (435.5, 1946)	1188.5 (366, 2495.5)
P/F ratio at baseline (mmHg)	364.9 (174.0)	328.7 (134.3)
Intravenous fluid before randomization (ml)		
None	40 (83%)	39 (79%)
200	5 (10%)	5 (10%)
201-500	3 (6%)	5 (10%)
Site of infection		
Respiratory tract	23 (48%)	19 (40%)
Urinary tract	10 (21%)	10 (21%)
Intraabdominal	10 (21%)	10 (21%)

Bloodstream	2 (4%)	1 (2%)
Central nervous system	0	1 (2%)
Other/Unknown	3 (6%)	7 (15%)
Baseline syndecan-1 level (ng/ml)**		
Median (Q1, Q3)	205.4 (136.2, 377.7)	221.7 (126.3, 758.9)
Geometric mean (95% CI)	258 (179.4 - 373)	312 (217 - 451)

Data indicate the mean (SD), median (Q1, Q3), or n (%) unless otherwise stated.

APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; LVEF: Left ventricular ejection fraction;

*Six data points are missing in the standard-rate group, and four are missing in the limited-rate group due to administrative reasons.

**One data point is missing in the limited infusion rate group due to administrative reasons.

4.2 Treatments during 6-hour intervention period

During the intervention period, the fluid administered in the limited-rate group was less than that of the standard-rate group. (39 ml/kg IQR 35 – 52 ml/kg vs. 53 ml/kg IQR 46-64 ml/kg; $p < 0.001$) (Figure 7). Patients in the limited rate group were less likely to received vasopressors (17% vs. 42%; $p = 0.007$) when compared with the standard-rate group. There was no difference in the vasopressor dose between the groups (Figure 8). The use of mechanical ventilation was less frequent in the limited-rate group than that of in the standard rate group (20% vs. 41.3%: $p = 0.049$) (Figure 9). The use of corticosteroid was comparable in both groups (8% vs. 10% $p = 0.73$). There was no difference in the use of albumin or time to antibiotics (Table 4). There were no differences between the hemodynamic data of patients in each group during the intervention and at 6 hours (Table 5).

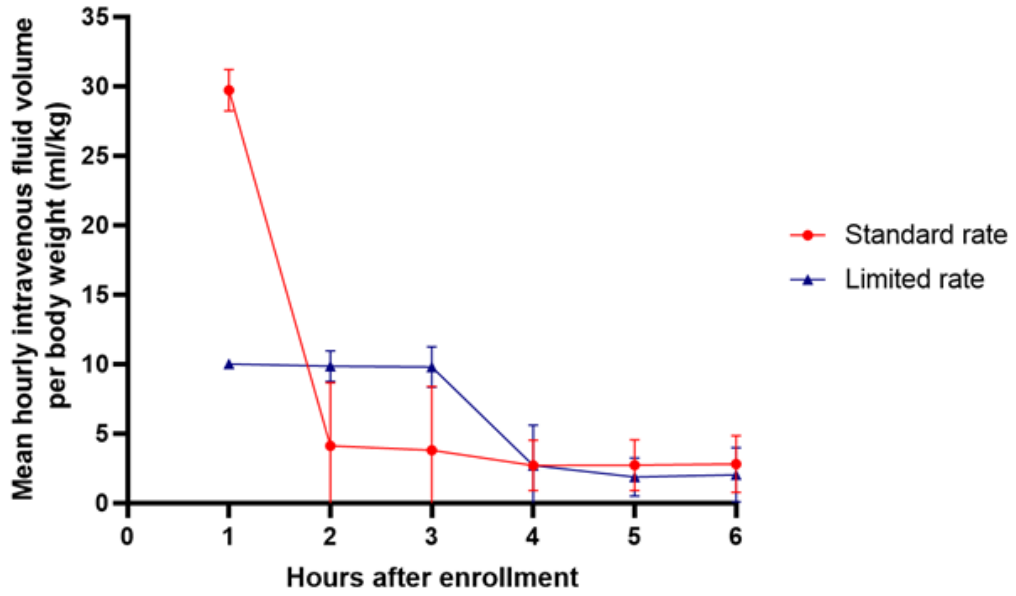


Figure 7 Mean hourly intravenous fluid volume per body weight (ml/kg) during the 6-hour intervention period. The error bars represent the standard deviation.

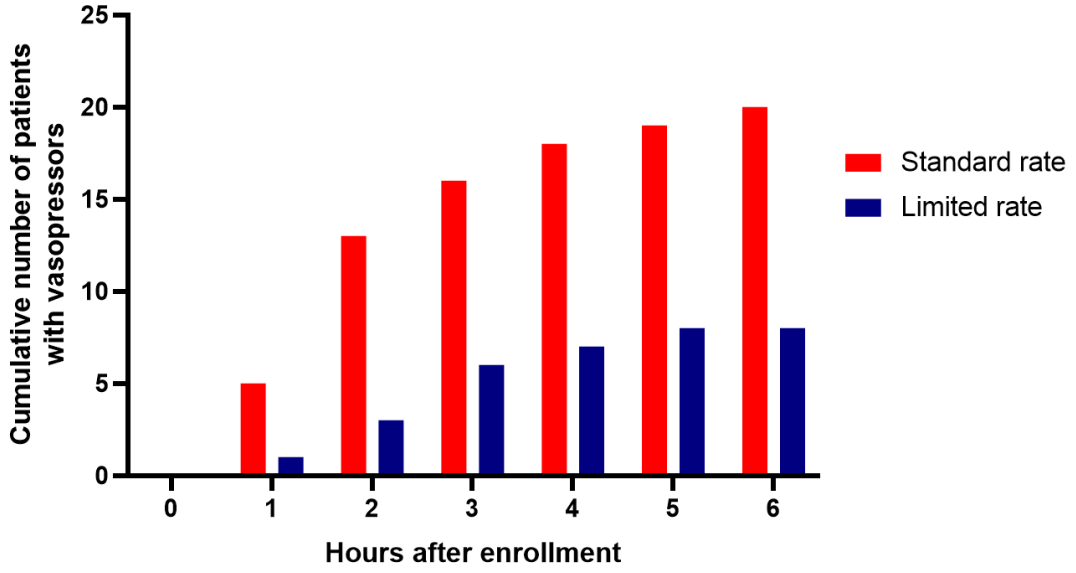


Figure 8 Cumulative number of patients with the need of vasopressors during the 6-hour intervention period

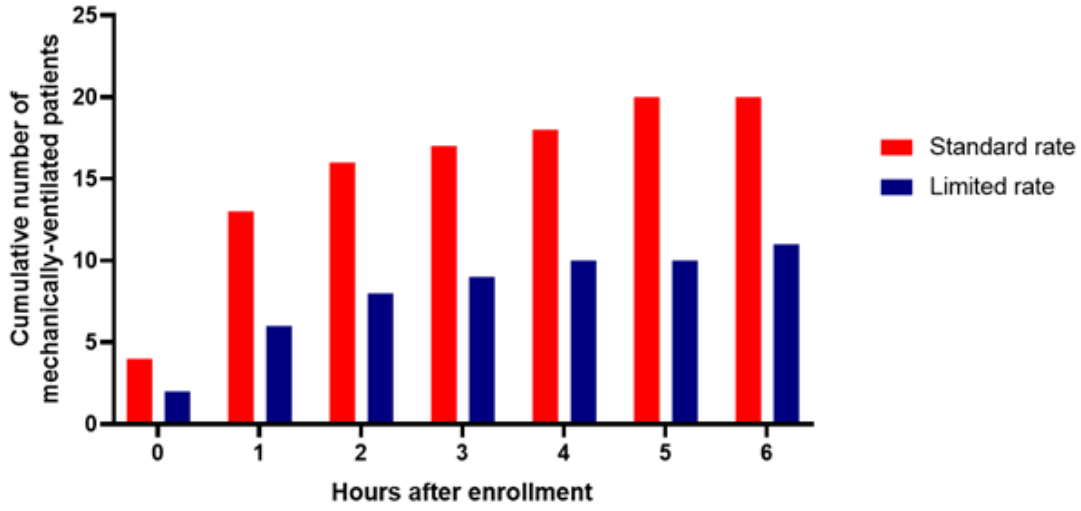


Figure 9 Cumulative number of mechanically-ventilated patients during the 6-hour intervention period.

Table 4 Treatments during the 6-hour intervention period.

	Standard rate (n = 48)	Limited rate (n = 48)	p value
Vasopressor use	20 (42%)	8 (17%)	0.007
Mechanical ventilation	20 (42%)	11 (23%)	0.049
Steroid use	5 (10%)	4 (8%)	0.73
Albumin use	2(4%)	2(4%)	> 0.99
Time to antibiotics from triage (minutes)	42 (30.5, 57.5)	49 (39, 66)	0.06

Data are n (%) and median (Q1, Q3).

Table 5 Hemodynamic data of the patients during the intervention

	Standard rate (n= 48)			Limited rate (n = 48)		
	Hour 0	Hour 1	Hour 6	Hour 0	Hour 1	Hour 6
SBP (mmHg)	105.9 (32.8)	115.9 (26.1)	113.0 (23.6)	114.3 (29.8)	117.4 (27.2)	116.8 (23.4)
DBP (mmHg)	58.4 (20.2)	62.4 (16.9)	65.3 (15.3)	66.5 (17.7) *	66.7 (16)	67.4 (13.2)
MAP (mmHg)	74.6 (22.7)	79.4 (19.2)	81.0 (16.7)	82.3 (19.9)	83.6 (18.5)	82.8 (15.9)
Heart rate (/min)	117.8 (28.1)	109.4 (23.3)	99.2 (18.1)	119.6 (24.3)	108.0 (23.2)	103.0 (18.9)
Respiratory rate (/min)	22.0 (20.0, 27.0)	20.0 (20.0, 22.0)	20.0 (18.0, 20.0)	22.0 (20.0, 25.0)	20.0 (20.0, 22.0)	20.0 (20.0, 22.0)
Oxygen saturation (%)	94.0 (87.5, 98.0)	99.0 (96.0, 100.0)	98.0 (97.0, 99.0)	96.0 (90.5, 98.0)	98.5 (97.0, 100.0)	98.0 (97.0, 100.0)

Data indicate the mean (SD) or median (Q1, Q3)

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure

*Significantly different from the hour-0 in the standard-rate group (p = 0.04)

4.3 Primary outcome

The geometric means of syndecan-1 in the standard rate (n=46) and the limited rate (n=44) groups are 265 ng/ml (95%CI 182 - 388 ng/ml) and 301 (95%CI 206 - 442 ng/ml) at baseline and 293 ng/ml (95%CI 209 – 410 ng/ml) and 273 (95%CI 183 - 408 ng/ml) at 6 hours, respectively. There was no significant difference in changes of the syndecan-1 level at 6 hours (GMR in the limited rate group, 0.82; 95% CI, 0.66 – 1.02; p = 0.07) (Figure 10). When the data was adjusted for the difference in baseline and treatment (hemodynamic status and vasopressor use within a 6-hour period) the difference remained insignificant (GMR in the limited rate group, 0.80; 95%CI 0.64 – 1.00; p = 0.05). According to the per-protocol analysis (42 patients in the standard-rate and 38 patients in the limited-rate group), there was no difference between the groups (GMR in the limited rate group, 0.84 95% CI (0.66 – 1.06; p = 0.07).

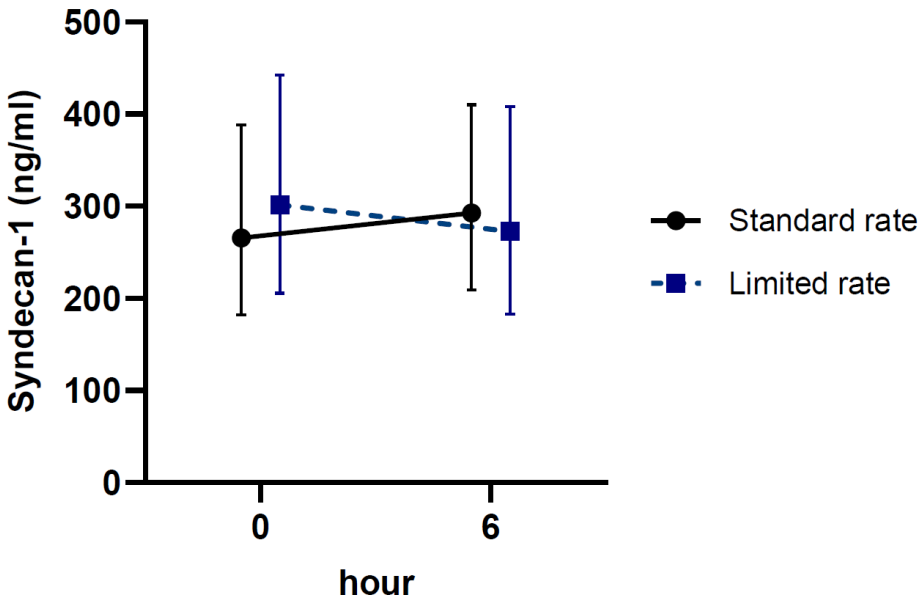


Figure 10 Changes in syndecan-1 levels from baseline to 6 hours. (Data are presented as the geometric mean, and error bars represent 95% confidence interval.)

4.4 Protocol Adherence

Protocol adherence was high in both groups since the termination of the protocol occurred in only six patients (12%) in the limited-rate group and five patients (10%) in the standard-rate group. The reasons for protocol termination were the physicians' decision to change the type of intravenous fluid; the patients exhibited signs of fluid overload; and the physicians' decision to increase the intravenous fluid rate (Figure 6).

4.5 Adverse events during intervention

There was one adverse event of cardiogenic pulmonary edema in the standard- rate group and two in the limited-rate group. There was no new arrhythmia or extravasation of the peripherally-administered vasopressors in both groups.

In the standard-rate group, one participant deceased during the intervention period due to life-threatening massive hemoptysis. This fatal event was reviewed and reported to the ethics committee and considered unrelated to the intervention.

4.6 Secondary outcomes

Regarding the physiologic parameters, there were no significant different changes of 6-hr lactate clearance, the proportion of patients with MAP ≥ 65 mmHg at 1 hour and 6 hours, the proportion of patients with shock reversal or the P/F ratio at 6 hours. The fluid input per body weight at 24 hours was lower in the limited-rate group, but at 72 hours, the volume of fluid used was comparable in both groups. There was no difference between groups in fluid balance at 24 or 72 hours, differences in organ failure-free days and hospital length of stay. The 90-day mortality was 18.8% and 31.3% in the limited-rate group and standard-rate group, respectively (relative risk in the limited rate group 0.67 (95%CI, 0.60 – 1.24; p = 0.16). The data was summarized in Table 6. The time to event analysis regarding 90-day mortality showed no difference between the two groups (hazard ratio in the limited rate group 0.55 (95%CI, 0.24 – 1.27; p = 0.16) (Figure 11).

Table 6 Secondary outcomes

Outcome	Standard rate (n = 48)	Limited rate (n = 48)	Point estimates (95% CI)*	p value
6-hr lactate clearance (%)	(n=46) 26.8 (39.8)	(n=46) 26.4 (38.1)	mean difference -0.5% (-16% to 15.6%)	0.95
Patients with MAP ≥ 65 mmHg at 1 hour	36/47 (77%)	42/47 (89%)	RR 1.17(0.97-1.41)	0.049
Patients with MAP ≥ 65 mmHg at 6 hours	43/47 (92%)	43/48 (90%)	RR 0.98(0.86 – 1.12)	0.73
P/F ratio at 6 hours (mmHg)	(n=46) 337 (178)	(n=46) 363 (159)	Mean difference 26(-44 to 96)	>0.99
Patients with shock reversal in 6 hours	34/47 (72%)	31/48 (65%)		0.42
Fluid input in 6 hours (ml)	(n=47) 2600 (2100, 3489)	(n=48) 2238(1898, 2488)		0.003

Fluid input per body weight in 6 hours (ml/kg)	(n=47) 53 (46, 64)	(n=48) 39 (35, 52)		<0.001
Fluid input per body weight in 24 hours (ml/kg)	(n=40) 115 (86, 146)	(n=45) 88 (63, 111)		0.02
Fluid balance in 24 hours (ml)	(n=40) 3758 (1237, 4975)	(n=43) 2896 (1520, 4535)		0.68
Fluid input per body weight in 72 hours (ml/kg)	(n=37) 175 (124, 220)	(n=37) 150 (108, 229)		0.70
Fluid balance in 72 hours (ml)	(n=37) 3140 (377, 5524)	(n=36) 4100 (2636, 7090)		0.13
Requirement for vasopressors	27/42 (64%)	20/46 (43%)	RR 0.68 (0.45 – 1.01)	0.05
Days alive and free from vasopressors up to 28 days	(n = 44) 26 (0, 28)	(n= 46) 27.5 (22, 28)		0.13
Requirement for mechanical ventilation	21/42 (50%)	19/46 (41%)	RR 0.83 (0.52 – 1.31)	0.41

Days alive and free from mechanical ventilation up to 28 days	(n=44) 27.5 (0, 28)	(n=46) 27 (9, 28)		0.91
Requirement for new RRT	4/42 (10%)	6/46 (13%)	RR 1.34 (0.41 – 4.52)	0.74
Days alive and free from RRT up to 28 days	(n=44) 28 (0, 28)	(n=46) 28 (21, 28)		0.60
Days alive and free from organ failure up to 28 days	(n=44) 25 (0, 27.5)	(n=46) 26 (9, 28)		0.37
Hospital LOS (day)	6 (5, 14)	11 (3.5, 25)		0.23
28-day mortality	12/48 (25%)	8/48 (17%)	RR 0.67 (0.30-1.48)	0.32
90-day mortality	15/48 (31%)	9/48 (19%)	RR 0.60 (0.29 – 1.24)	0.16

Data are mean (SD), n/total n (%) and median (Q1, Q3).

MAP: mean arterial pressure; P/F: PaO_2/FiO_2; RR: Relative risk; RRT: renal replacement therapy; LOS: length-of-stay

*Point estimates are for the limited-rate group compared to the standard-rate group.

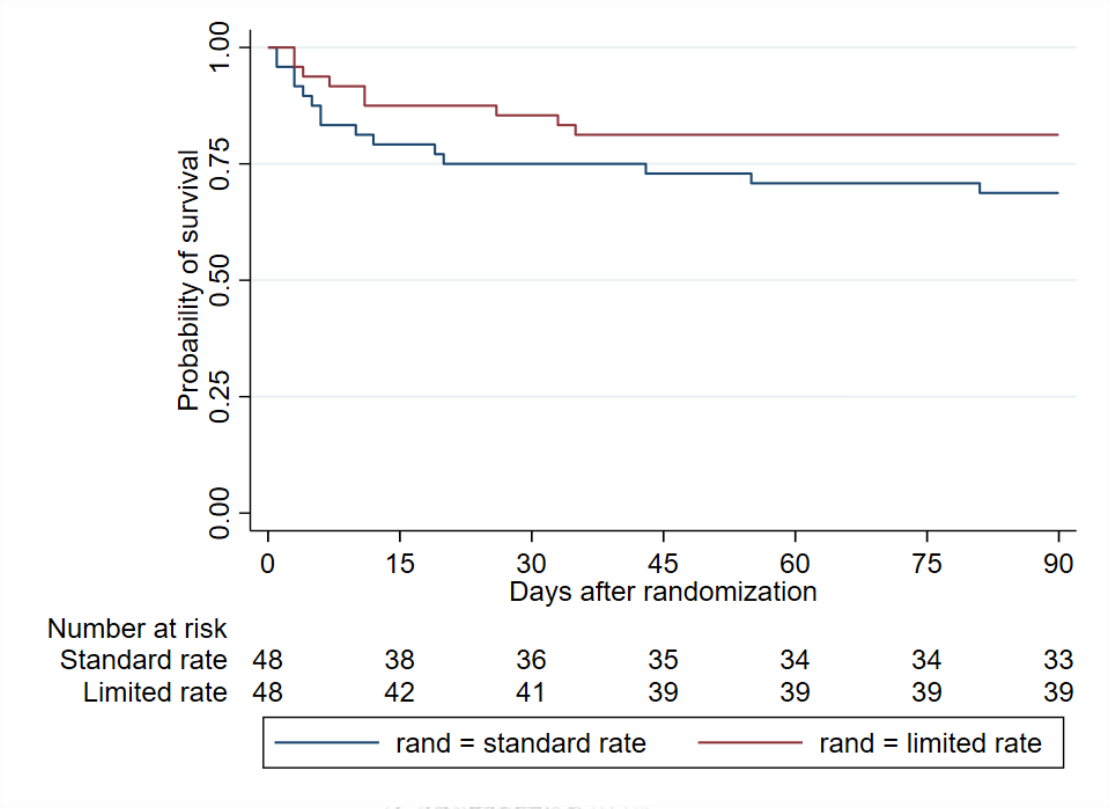


Figure 11 Kaplan-Meier survival graph of the patients

4.7 Subgroup analysis

No significant difference was found regarding the effect of limited rate according to the prespecified subgroups; baseline syndecan-1, NT-proBNP, lactate and APACHE II score (p = 0.14 to 0.50 for interaction) (Figure 12).

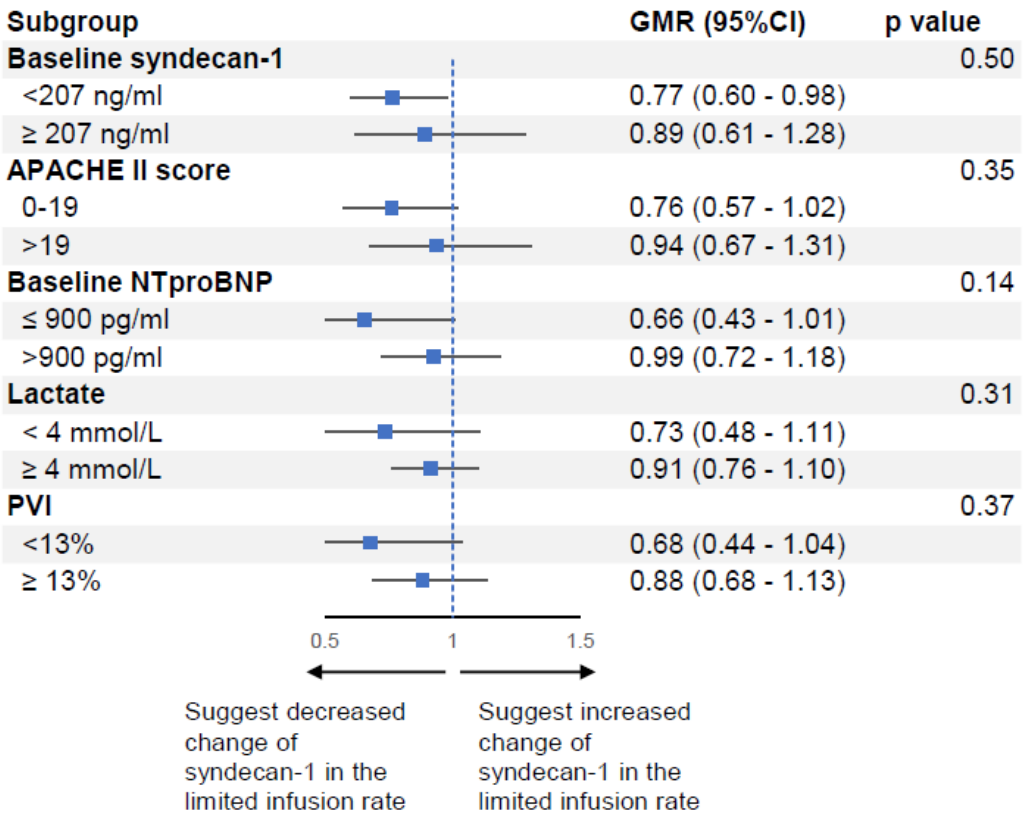


Figure 12 Prespecified subgroup analysis. P-value for the interaction. (GMR: Geometric mean ratio; CI: Confidence interval; APACHE: Acute Physiology and Chronic Health Evaluation; NT-proBNP: N-terminal pro-b-type natriuretic peptide, PVI: Pleth variability index)

4.8 Post-hoc analysis of outcomes in patients with early norepinephrine administration

The total of 8 out of 96 patients (8%) were classified as having early norepinephrine administration. Compared to those without, patients with early norepinephrine administration had lower baseline MAP (mean difference -21 mmHg, 95%CI -36 to -6 mmHg, p = 0.007), lower hemoglobin level (mean difference -2.1 g/dl, 95% CI – 4.1 to -0.4 g/dl, p = 0.046), higher total bilirubin level (mean difference 2.9 g/dl, 95% CI 1.2 to 4.7 g/dl, p = 0.001), and higher SOFA score (median 7, IQR 6.5-7 versus median 4, IQR 2-6, p <0.001).

The fluid administration and fluid balance did not significantly differ between the groups with and without early norepinephrine. The clinical outcomes, including organ failure outcomes and mortality also did not differ between the two groups (Table 7).

Table 7 Comparing clinical outcomes between the patients who received early and late norepinephrine

Outcomes	Late norepinephrine (n = 88)	Early norepinephrine (n = 8)	P value
Fluid input in 6 hours (ml)	(n=87) 2350 (2000 -2900)	3100 (2400-4435)	0.045
Fluid input in 24 hours (ml)	(n=77) 5107 (3565-6798)	5914 (4809-6837)	0.37
Fluid balance in 24 hours (ml)	(n=75) 3380 (1500-4964)	3189 (640-3797)	0.4
Fluid input in 72 hours (ml)	(n=66) 7993(6230-11511)	8941(7656-11168)	0.5
Fluid balance in 72 hours (ml)	(n=65) 3846(2022-6882)	3204 (210-5701)	0.38
Days alive and free from vasopressors up to 28 days	(n=82) 27 (20-28)	26 (25-26.5)	0.41
Days alive and free from mechanical ventilation up to 28 days	(n=82) 26(0-28)	28(26-28)	0.082

Days alive and free from RRT up to 28 days	(n=82) 28 (0-28)	28 (28-28)	0.045
Days alive and free from organ failures up to 28 days	(n=82) 25 (0-28)	24 (22.5-26)	0.98
Hospital free day up to 90 days	74 (0-83)	78 (28.5-83.5)	0.91
28-day mortality	20 (23%)	0 (0%)	0.13
90-day mortality	22 (25%)	2 (25%)	>0.99

Data are mean (SD), n/total n (%) and median (Q1- Q3).

RRT: renal replacement therapy

4.9 Post-hoc analysis regarding association of syndecan-1 levels with clinical outcomes.

4.9.1 Baseline characteristics of the participants

We included 95 adults with sepsis-induced hypoperfusion who presented to the ED with syndecan-1 level measurements at baseline (Figure 13). The mean age of patients was 71 years old, 36 (38%) were female. Almost all patients (96%) reported at least one underlying disease. The median Charlson Comorbidity Index of this cohort was 5 (IQR 4-7). Almost half (43%) of patients suffered from a pulmonary source of infection. There were 24 (25%) non-survivors at 90 days. The baseline characteristic of the participants as stratified by survival are presented in table 8. Generally, non-survivors had a higher baseline severity index as measured by APACHE II and SOFA score and had lower pH on arterial blood gas on admission. There was no significant difference in the proportion of patients diagnosed with septic shock at 6 hours in survivors and

non-survivors. Nonsurvivors received significantly larger volumes of fluid in 24 hours (median 6.1 (IQR 4.4-7.6) L versus 4.8 (IQR 3.2 – 6.6) L, p = 0.03) and 72 hours (median 11.5 (IQR 6.9 – 12.6) L vs 7.8 (IQR 5.83 – 10.6) L, p = 0.03) than survivors.

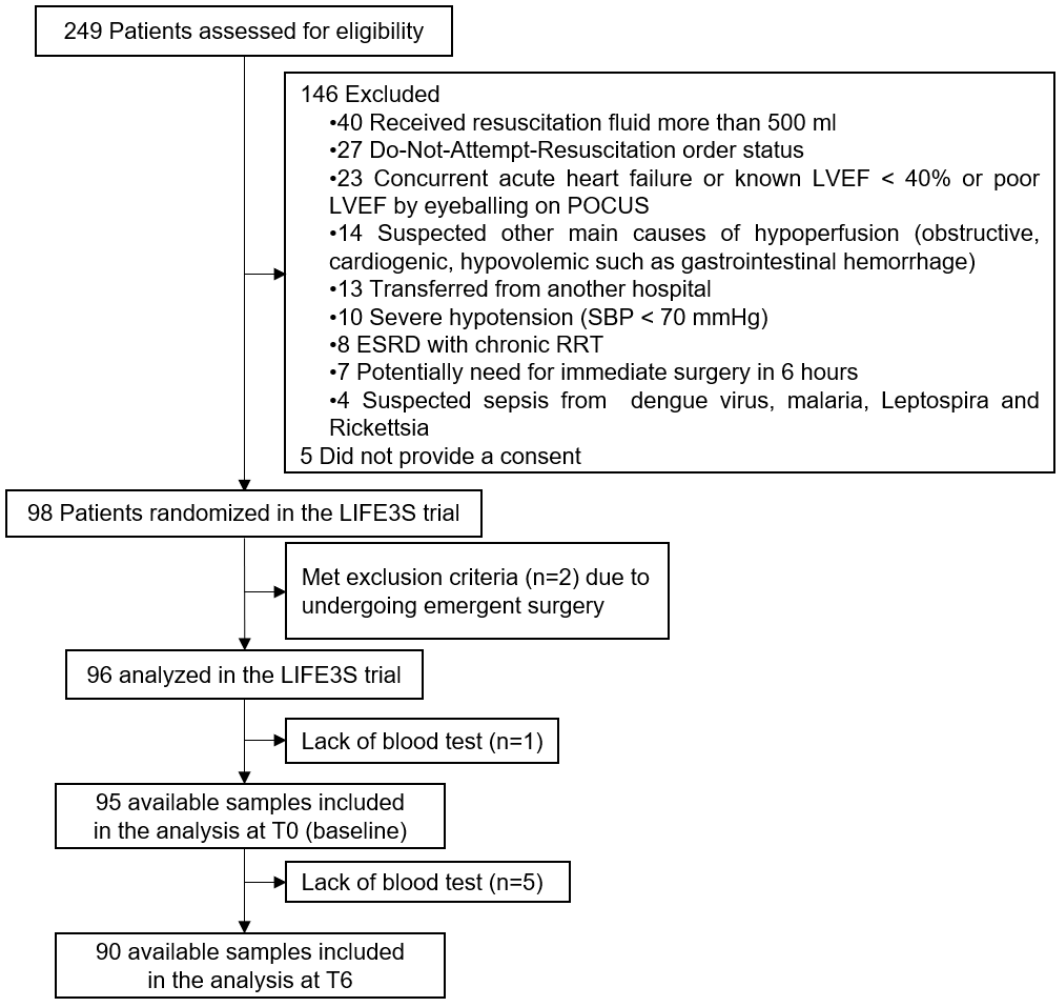


Figure 13 Flow diagram of enrollment and analysis. LVEF: left ventricular ejection fraction; POCUS: point-of-care-ultrasound; SBP: systolic blood pressure; ESRD: end-stage renal disease; RRT: renal replacement therapy.

Table 8 Baseline characteristics of the participants in the post-hoc analysis

	Data available	All patients N = 95	90-day Survivors N = 71	Non-survivors N = 24	p-value
Age (year)	95	76(65-83)	76 (65-82)	77.5 (67-83)	0.67
Female	95	36 (38%)	26 (37%)	10 (42%)	0.66
Body weight (kg)	95	52.1 (10.4)	52.3 (11.1)	51.5 (8.0)	0.76
APACHE II	95	18.0 (12.0-23.0)	17.0 (11.0-21.0)	20.0 (13.5-27.0)	0.05
SOFA	95	4.0 (2.0-6.0)	4.0 (2.0-6.0)	5.0 (4.0-7.0)	0.007
Hypoperfusion defined by:					
Lactate ≥ 2 mmol/L	95	83 (87%)	60 (85%)	23 (96%)	0.15
Hemodynamic instability	95	36 (38%)	30 (42%)	6 (25%)	0.13
Charlson Comorbidity Index	95	5.0 (4.0-7.0)	5.0 (4.0-7.0)	5.0 (4.0-6.5)	0.68
Presence of underlying diseases	95	89 (96%)	65 (94%)	24 (100%)	0.23
Cerebrovascular disease	95	53 (56%)	41 (58%)	12 (50%)	0.51
Diabetes mellitus	95	41 (43%)	33 (46%)	8 (33%)	0.26
Malignancy	95	32 (34%)	21 (30%)	11 (46%)	0.15

Ischemic heart disease	95	11 (12%)	9 (13%)	2 (8%)	0.57
Chronic kidney disease	95	6 (6%)	5 (7%)	1 (4%)	0.62
Currently use systemic steroid	95	16 (17%)	10 (14%)	6 (25%)	0.22
Previous hospitalization within 90 days prior to this hospital visit	95	41 (43%)	31 (44%)	10 (42%)	0.86
Previous antibiotic treatment within 30 days prior to this hospital visit	95	27 (28%)	18 (25%)	9 (38%)	0.25
Time to antibiotic from triage (min)	95	53.3 (28.7)	55.3 (30.4)	47.6 (22.4)	0.26
Site of infection					0.37
Respiratory tract	95	41 (43%)	28 (39%)	13 (54%)	
Urinary tract	95	20 (21%)	15 (21%)	5 (21%)	
Intraabdominal	95	20 (21%)	16 (23%)	4 (17%)	
Bloodstream	95	3 (3%)	3 (4%)	0 (0%)	
Central nervous system	95	1 (1%)	1 (1%)	0 (0%)	
Other/Unknown	95	10(10%)	8 (11%)	2(8%)	

Diagnosis of septic shock at 6 hours	92	17 (18%)	11 (16%)	6(27%)	0.22
pH	95	7.44 (0.06)	7.45 (0.05)	7.41 (0.08)	0.02
Lactate (mmol/L)	95	4.1 (2.7-5.4)	4.1 (2.5-5.1)	4.4 (3.0-8.9)	0.07
Cumulative volume of fluid administration (L)					
6 hours	94	2.4 (2-2.9)	2.3(1.9-2.9)	2.5 (2.1-3)	0.32
24 hours	84	5.2 (3.6-6.7)	4.8 (3.2-6.6)	6.1 (4.4-7.6)	0.03
72hours	73	8.2 (6.2-11.5)	7.80 (5.83-10.64)	11.5 (6.9-12.6)	0.03

Data indicate the mean (SD), median (Q1, Q3), or n (%) unless otherwise stated. APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: sequential organ failure assessment

4.9.2 Correlations between syndecan-1 at T0 and patients' age, comorbidities, or sepsis severity.

At T0, the syndecan-1 level at hour 0 ranged from 12 ng/ml to 12850 ng/ml with a median level of 207 (IQR 135-438) ng/ml. At T6, from the available 90 samples, the syndecan-1 level ranged from 35 ng/ml to 12880 ng/ml with a median level of 207 (IQR 128-490) ng/ml. In the same patients, there was no significantly different change from T0 ($p = 0.47$); the levels were increasing in 46 out of 90 patients (51%) and decreasing in 44 patients (49%).

We assessed the correlation between the syndecan-1 level at T0 with baseline characteristics of patients. We found negative correlation between syndecan-1 level and patients' age ($\rho = -0.23$, $p = 0.03$) and positive correlation between syndecan-1 and sequential organ failure assessment (SOFA) score ($\rho = 0.35$, $p < 0.001$). When analyzed

to the component of the SOFA score, there were significant positive correlations with liver component (ρ = 0.36, p < 0.001) and coagulation component (ρ = 0.26, p = 0.01). No correlation was observed between syndecan-1 and Charlson Comorbidity Index (ρ = 0.1, p = 0.34) or Acute Physiology and Chronic Health Evaluation (APACHE) II score (ρ = 0.14 p = 0.17).

4.9.3 Correlations between syndecan-1 and laboratory values

We explored the correlation between syndecan-1 at T0 with the baseline laboratory values. We observed no correlation between syndecan-1 with baseline white blood cell counts, creatinine, bicarbonate level or arterial pH. Positive correlations was found between syndecan-1 and bilirubin level (ρ = 0.34, p <0.001, n = 94) and negative correlations were found between syndecan-1 and platelet counts (ρ = -0.24, p = 0.02) , albumin (ρ = -0.32, p =0.002, n = 94) and PaO_2 (ρ = -0.25, p = 0.01, n = 93) While no correlation was found between syndecan-1 and lactate at T0, syndecan-1 at T6 correlated with lactate at T6 (ρ = 0.26, p = 0.01, n = 88).

4.9.4 Correlations of syndecan-1 and subsequent fluid administration

The syndecan-1 at T0 or T6 were not correlated with the volume of fluid administration at 6 hours. However, both syndecan-1 at T0 and T6 were correlated with the volume of fluid administration at 24 hours and 72 hours, as shown in Figure 14.

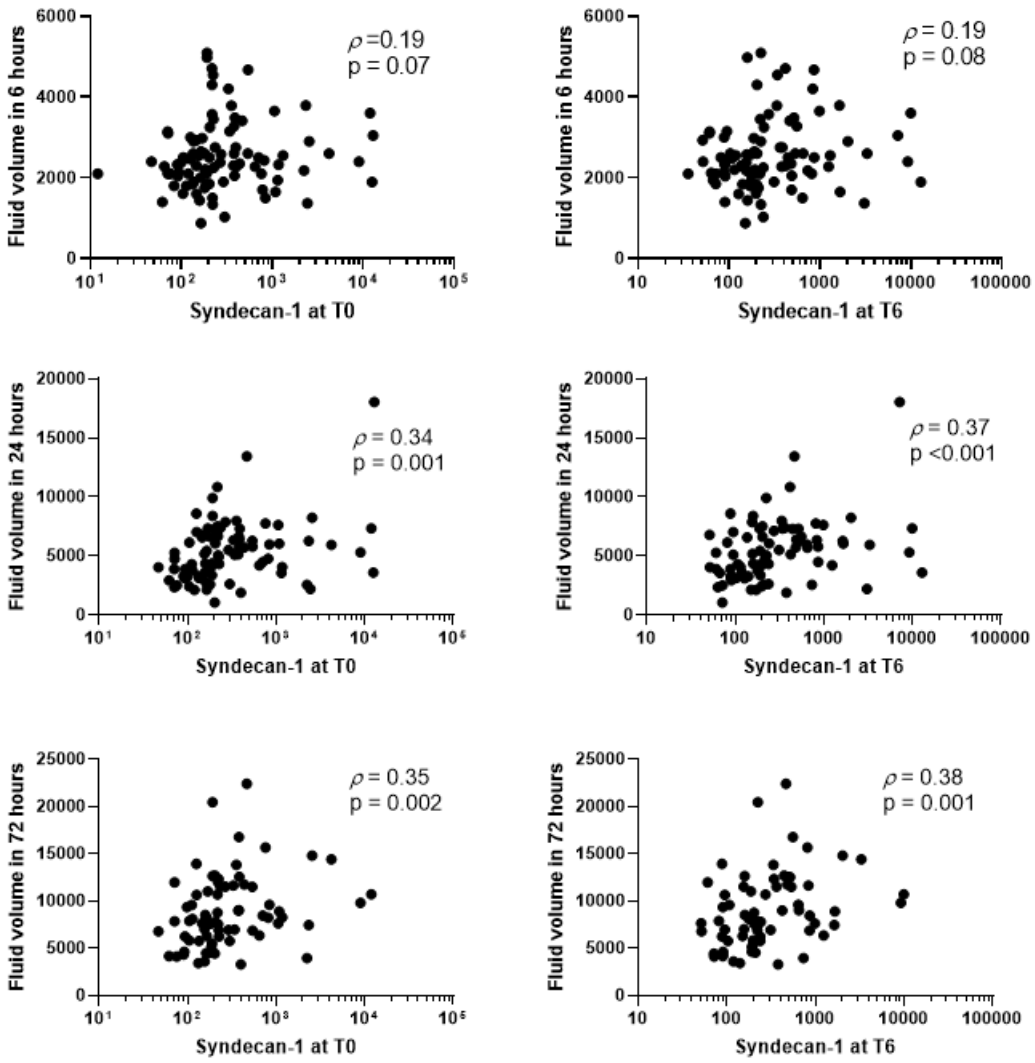


Figure 14 Correlations between the syndecan-1 level at T0, T6 and cumulative fluid volume at 6, 24 and 72 hours.

Regarding fluid balance and fluid output, there was no correlation between the syndecan-1 level at T0 or T6 and fluid balance or fluid output at the three time points. For the exploration regarding the pathophysiology of glycocalyx shedding, we found that syndecan-1 was not correlated with NT-proBNP, both at T0 (ρ = 0.18, p = 0.09, n = 86) and T6 (ρ = 0.19 p = 0.08 n= 83). We also observed no correlation between volume of fluid administration at different time points and baseline NT-proBNP or changes of NT-probBNP in 6 hours.

4.9.5 Association of syndecan-1 and clinical outcomes.

During the first 6 hours, 17 out of 92 patients (18%) were diagnosed with septic shock. We found that both the syndecan-1 level at T0 and T6 in patients with septic shock were significantly different from patients without septic shock. At T0, the median syndecan-1 level between patients with septic shock and without septic shock was 375 (IQR 192 – 707) ng/ml and 196 (IQR 127 – 398) ng/ml, p = 0.03. Syndecan-1 at T6 were also higher in patient with septic shock (median 393 (IQR 202-861) ng/ml versus those without 192 (IQR 105 – 485) ng/ml, p = 0.02. (Figure 15)

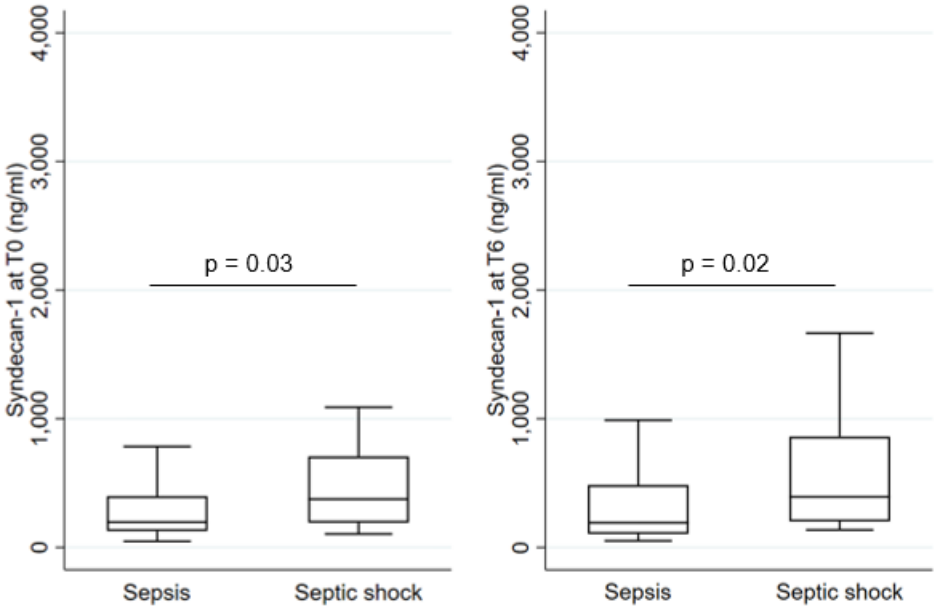


Figure 15 Box plots showing the median and interquartile range of syndecan-1 levels at T0 and T6 in patients with sepsis versus septic shock.

There were 46 out of 87 (53%) patients that require vasopressor during the first 28 days of hospital stay. The maximum dose of vasopressor as measured in norepinephrine equivalent was from 0.027 mcg/kg/min to 2.13 mcg/kg/min with the median value of 0.12 (IQR 0.07 – 0.27) mcg/kg/min. No significant difference in syndecan-1 concentration was found between patients who required or did not require vasopressors (T0: median 220 (IQR 156 – 467) ng/ml versus 189 (IQR 127 – 389) ng/ml, p = 0.24; T6: median 224 (IQR 159 – 556) ng/ml versus 173 (IQR 90 – 380) ng/ml, p = 0.05). In patients with vasopressor requirement, both syndecan-1 levels at T0 and T6 were correlated with maximum dose of vasopressor (T0: ρ = 0.45, p = 0.002, n = 46 and T6: ρ = 0.43, p = 0.004, n = 43)

There were 39 out of 87 patients (45%) that was intubated during the first 28 days of hospital stay. No significant difference in the syndecan-1 level was found between patients who required or did not require intubation (T0: median 218 (IQR 133 – 389) ng/ml versus 196 (IQR 135 – 746) ng/ml, p = 0.9; T6: median 288 (IQR 165 – 487) ng/ml versus 195 (IQR 94 – 652) ng/ml, p = 0.2). No correlation was found between the duration of mechanical ventilation and the syndecan-1 level (T0: ρ = 0.09, p = 0.59, n = 38; T6: ρ = -0.04, p = 0.82, n = 35).

There were 10 out of 87 patients (11%) that require new renal replacement therapy (RRT) during the first 28 days of hospital stay. Higher syndecan-1 concentration at T0 was observed in patients with RRT requirement than those without (median level 453 (IQR 217 – 2554) ng/ml versus 192 (IQR 127 – 381) ng/ml; p = 0.008). Also, at T6, a higher syndecan-1 level was observed in patients with RRT requirement than those without (median level 474 (IQR 380 – 2036) ng/ml versus 195 (IQR 105 – 466) ng/ml; p = 0.007) (Figure 16).

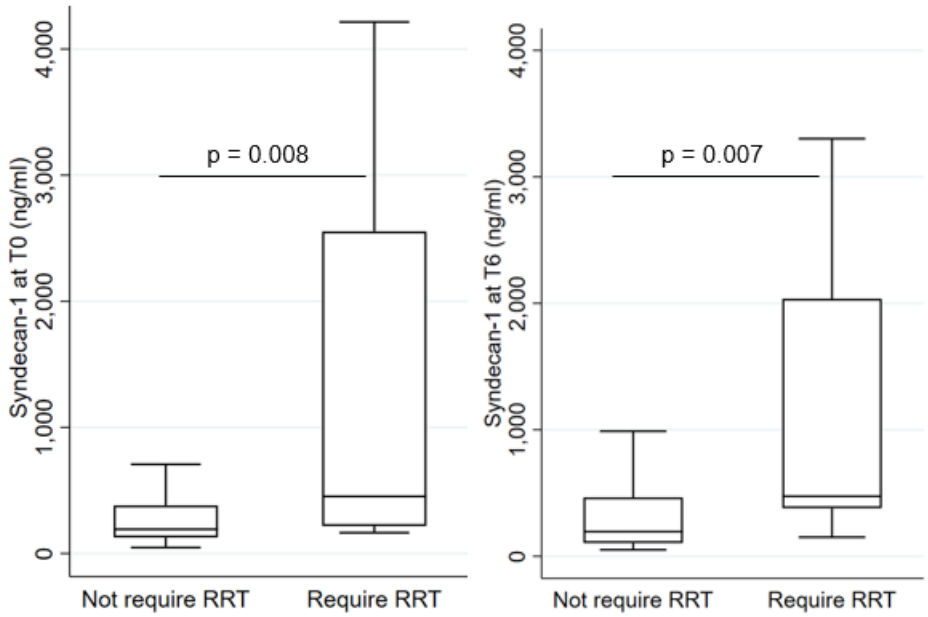


Figure 16 Box plots showing the median and interquartile range of syndecan-1 levels at T0 and T6 in patients who required and did not require renal replacement therapy (RRT)

4.9.6 Association of syndecan-1 level and mortality

Median syndecan-1 level at T0 was higher in non-survivors at 90 day (387 (IQR 190 – 746) ng/ml) than in survivors (189 (IQR 126 – 375) ng/ml), p = 0.02. At T6, higher median syndecan-1 was also observed in non-survivors at 90 days (483(IQR 192 – 860) ng/ml) than in survivors (187 (IQR 95– 363) ng/ml), p = 0.003. (Figure 17) After stratifying syndecan-1 levels by quartile, we found that a higher syndecan-1 level at T6 was significantly associated with higher risks of 90-day mortality (Figure 18).

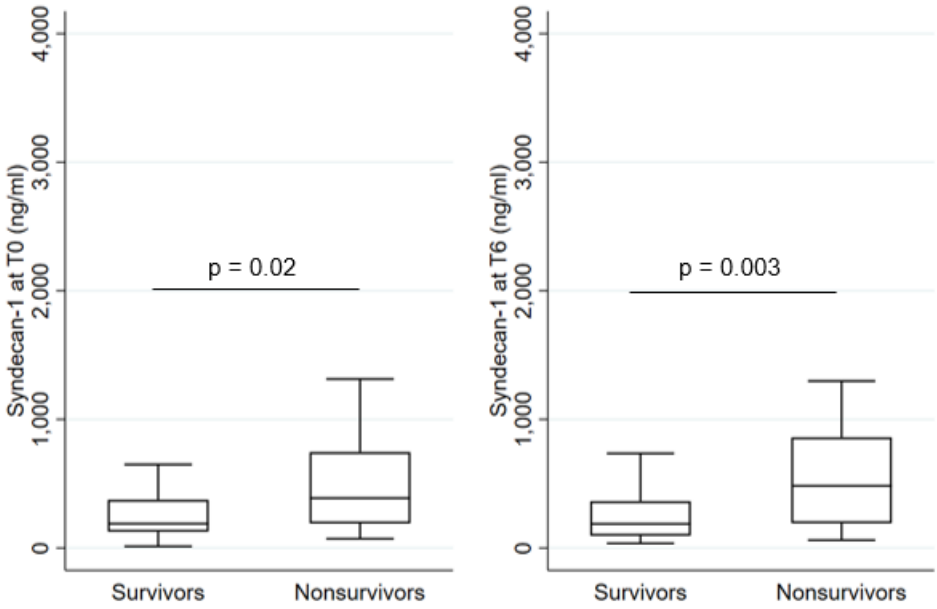


Figure 17 Box plots showing the median and interquartile range of syndecan-1 levels at T0 and T6 in survivors and non-survivors.

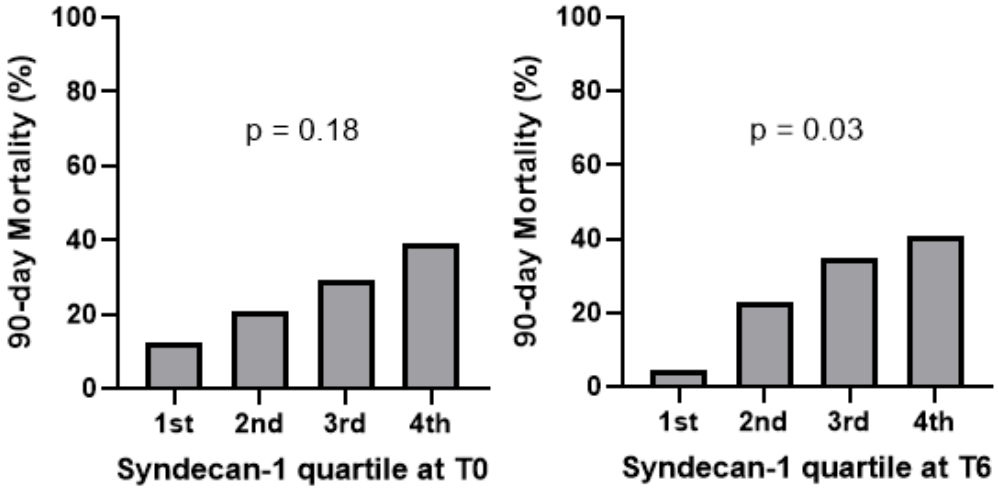


Figure 18 Higher syndecan-1 levels by quartile at T6 were associated with 90-day mortality.

When analyzed using the trends of the syndecan-1 level, the mortality risk was not different between those who had increasing when compared with decreasing level from T0 to T6 (RR 1.24 95%CI 0.61 – 2.54; p = 0.55).

4.9.7 Prediction of mortality

We further explore the predictive ability of syndecan-1. The AUROC of the syndecan-1 level at T0 in predicting 90-day mortality was 0.66 (95%CI 0.54 – 0.79). The best cut-off point for the syndecan-1 level was at 383 ng/ml with a sensitivity of 54% and a specificity of 79% in identifying septic patients with 90-day mortality. The AUROC of the syndecan-1 concentration at T6 in predicting 90-day mortality was 0.71 (95%CI 0.59 – 0.83). The best cutoff point for the syndecan-1 level was also at 383 ng/ml with a sensitivity of 65% and a specificity of 78% in identifying septic patients with 90-day mortality.

When categorizing syndecan-1 level into high and low level according to the optimum cutoff point, high syndecan-1 level both at T0 and T6 was significantly associated with 90-day mortality (OR 4.41 (95%CI 1.65 – 11.8) p= 0.003, OR 6.5 (95%CI 2.31 – 18.35) p < 0.001 respectively). Multivariable logistic regression shown that high syndecan-1 level both at T0 and T6 are independent predictors of mortality (Table 8) Higher AUROC, when compared with the model using syndecan-1 level alone, was observed in both models. The AUROC of the model 1 (high syndecan-1 level at T0, SOFA score, MAP < 65 mmHg and Hemoglobin level) is 0.832 (95%CI 0.734 – 0.929). The AUROC of the model 2 (high syndecan-1 level at T6, SOFA score and MAP < 65 mmHg) is 0.844 (95%CI 0.74 – 0.95). (Figure 19).

Table 9 Univariable and multivariable logistic regression of factors in prediction of 90-day mortality

Characteristics	Univariable analysis	Multivariable analysis				
		Model 1: Syndecan-1 level at T0		Model 2: Syndecan-1 level at T6		
	Crude OR (95%CI)	p value	Adjusted OR (5%CI)	p value	Adjusted OR (5%CI)	p value
High Syndecan-1 level at T0 (> 383 ng/ml)	4.41 (1.65 - 11.8)	0.003	3.57 (1.12 - 11.34)	0.03		
High Syndecan-1 level at T6 (> 383 ng/ml)	6.5 (2.31 - 18.25)	<0.001			5.62 (1.71 - 18.47)	0.004
Glasgow coma score < 15	2.28 (0.88 - 5.85)	0.09				
SOFA score (	1.29 (1.06 - 1.57)	0.012	1.42 (1.08 - 1.85)	0.009	1.57 (1.14 - 2.14)	0.005
Lactate (by 1 mmol/L)	1.22 (1.04 - 1.45)	0.01				
APACHE II score	1.08 (1.00 - 1.16)	0.04				
Hemoglobin (by 1 g/dl)	0.85 (0.71 - 1.01)	0.07	0.77 (0.63 - 0.96)	0.02		
pH (by unit of 0.1)	0.44 (0.2 - 0.92)	0.03				
MAP < 65 mmHg	0.23 (0.06 - 0.85)	0.03	0.89 (0.01 - 0.43)	0.003	0.06 (0.01 - 0.36)	0.002

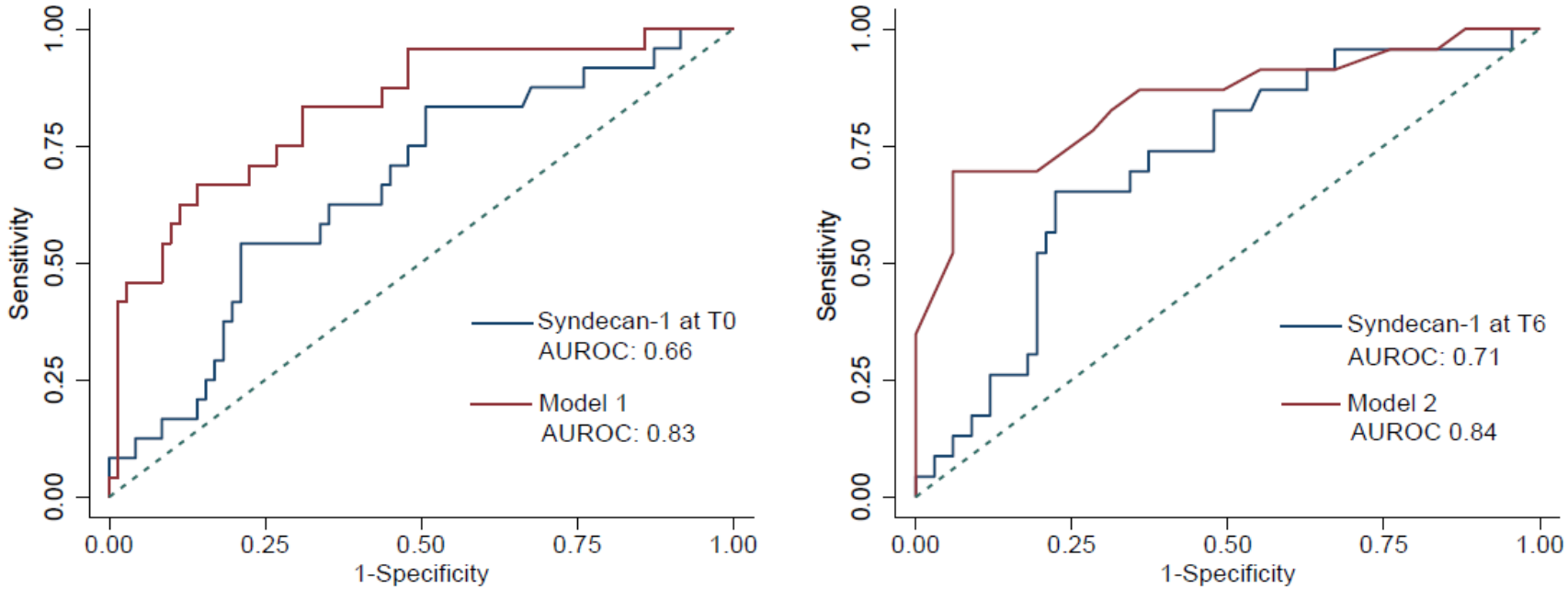


Figure 19 The area under the receiver operating characteristic curve (AUROC) for prediction of 90-day mortality.

Chapter 5 Discussion

In this randomized controlled trial of resuscitating patients with sepsis-induced hypoperfusion in the ED, we found the limited rate of fluid resuscitation, as compared with the standard rate, did not significantly reduce the syndecan-1 change at six hours. However, less amount of 6-hr and 24-hr fluid input volume was used in the limited rate group compared with the standard rate strategy. There was no significant difference in, organ failure outcomes, adverse event, and mortality rate.

Previous studies exhibited the association between hypervolemia from rapid fluid administration and glycocalyx shedding as measured by syndecan-1. In an animal model of sepsis, rapid fluid administration (30 ml/kg/hr) resulted in more syndecan-1 shedding compared to the slower rate (10 ml/kg/hr) [53]. In humans, the increased syndecan-1 level was detected after rapid fluid bolus in 15 minutes [16]. Higher level syndecan-1 were found after fluid bolus in healthy pre-operative patients, concurrently with higher level of atrial natriuretic peptide (ANP) [17]. Released in response to hypervolemia, the peptide hormone ANP and brain natriuretic peptide (BNP) was found to have in vivo activity of shedding glycocalyx [56]. Moreover, rapid fluid bolus could lead to shear stress that directly activate secretion of the matrix metalloproteinases from the endothelial cell and stimulate glycocalyx shedding [57]. Though limited rate strategy could mitigate transient hypervolemia and shear stress from fluid administration, our study did not show a significant reduction of syndecan-1 change in different fluid resuscitation strategies. This finding could be explained by the heterogeneity of septic patients, which resulted in the differences in patients' characteristics in this small randomized controlled trial since the destruction of endothelial glycocalyx can result from various factors such as the inflammation, hypoxia or vasopressor administration [41, 43, 58]. Moreover, syndecan-1 was proposed to be a biomarker in many diseases (e.g., diabetes mellitus, chronic kidney disease, lung cancer, hepatocellular carcinoma, breast cancer and hematologic malignancy) as it was discovered to be elevated in patients with those conditions [59]. In our study, after adjusting the difference in hemodynamic instability and vasopressor

administration, the effect of the limited-rate fluid strategy on the pre- and post-treatment reduction in syndecan-1 level was more pronounced but still marginally significant.

It is interesting that in the standard rate group, the use of vasopressor is more than that of the limited rate group. This would partly be explained by imbalance baseline of the participants; there were more patients with hemodynamic instability in the standard rate group that would require more vasopressor. Moreover, the proportion of mechanically ventilated patients in the standard-rate group was higher than that in the limited-rate group. This might also explain the increased use of vasopressors in the standard-rate group since mechanical ventilation potentially induced hemodynamic instability in preload-dependent patients [60]. However, previous studies also potentially provide the partial explanation from the evidence of ineffectiveness of rapid fluid bolus. In a volume kinetics study in human volunteer, the fraction of the infused crystalloid that remained in the plasma was higher for lower rate of infusion [26]. Another study found that the cardiac output increased for 0.02 L /min in slower fluid bolus (rate 500 ml/hr) when compared to rapid fluid bolus (rate 2000 ml/hr). The effect returned to baseline after infusion was completed [27].

Our study demonstrated that the limited rate strategy led to a reduction of fluid volume used in 6 and 24 hours without significant adverse events or any difference in the clinical outcomes. However, they were not adequately powered to detect the differences and thus should be considered as exploratory. The significant difference in the clinical outcomes was not demonstrated in the previous pilot studies of the limited volume of fluid resuscitation. In a pilot randomized study in an intensive care setting, patients with septic shock treated with the restrictive fluid approach received less fluid during the initial five days than those with the liberal strategy (absolute difference -1.2 L; 95% CI -2.0 to -0.4 L), and there was a signal towards mitigating kidney injury in restrictive fluid approach [61]. In the ED setting, the implementation of the limited volume of resuscitation coupled with early vasopressor was feasible and associated with a decreased amount of fluid in the initial phase of resuscitation [62]. The large study that compares the clinical outcomes of various fluid resuscitation approaches is currently conducted and potentially powered to determine their effects on the relevant outcomes [63].

For the post-hoc analysis, glycocalyx shedding as measured by syndecan-1 levels was significantly associated with sepsis severity, the diagnosis of septic shock, organ dysfunction (i.e., renal replacement therapy and vasopressor's dose), and mortality. Moreover, syndecan-1 had an acceptable discriminative ability to identify sepsis survivors.

Our findings corroborate results from previous studies that showed the association of syndecan-1 with organ failure and mortality. Regarding organ failure outcomes, high plasma syndecan-1 was associated with acute kidney injury and intubation requirement in studies conducted in EDs [48, 64]. The syndecan-1 levels at ICU admission were associated with risks of acute kidney injury [65] and septic shock [66]. In another study, the syndecan-1 level at day 2 in ICU was associated with the number of vasopressors required in the first 24 hours, diagnosis of ARDS in patients with non-pulmonary sepsis, and need for RRT [67]. Moreover, syndecan-1 was associated with mortality in both ED and ICU-based studies [51, 64, 65, 67-69]. Two ICU-based studies also reported good discriminative ability of syndecan-1 to predict sepsis mortality [50, 51]. In our study, the syndecan-1 level at 6 hours showed slightly better discriminative ability regarding mortality outcome than the level at study enrollment in the ED. We could imply that plasma syndecan-1 level after initial resuscitation or at the time of ICU admission could be adequate or even better in prognostication than at the time of ED admission.

Prior clinical studies demonstrated an uncertain association between glycocalyx damages and fluid administration in patients with sepsis. A large cohort study demonstrated no association between the syndecan-1 level at ICU admission and subsequent fluid administration on the first ICU day [65]. A similar volume of fluid administration in the ED was observed in both groups of high and low syndecan-1 levels [48]. However, another study showed that higher syndecan-1 quartile measured on the 2nd ICU day was associated with higher fluid balance [67]. Two studies in the ED suggested an association between the volume of fluid administration and glycocalyx shedding as measured by markers other than syndecan-1 [49, 70]. Our study found that the syndecan-1 level correlated with volume of subsequent fluid administration in 24 and 72 hours. We hypothesized that the degree of glycocalyx degradation reflects the

fluid requirement in the early resuscitation, as syndecan-1 was associated with higher sepsis severity and degree of endothelial damage. A similar association of syndecan-1 level with the 24-hr fluid requirement was also observed in patients with major burns [71]. Different approaches to shock stabilization and varying proportions of patients with hemodynamic instability in previous studies might explain the conflicting results.

Hypervolemia was proposed as a mechanism that induced glycocalyx shedding via the natriuretic peptide stimulation. Natriuretic peptides, secreted in response to myocardial wall stretch, had been shown to cleave glycocalyx in an experimental study [56]. A previous study in healthy volunteers showed that fluid administration increased Atrial Natriuretic Peptide (ANP) and increased glycocalyx shedding [17]. Similar to a previous study in an emergency department [72], we demonstrated increasing NT-proBNP levels after sepsis resuscitation. However, no association between NT-proBNP and syndecan-1 levels was observed, which is also comparable with a previous study that demonstrated no association of glycocalyx shedding with ANP or brain natriuretic peptide (BNP) [70]. We hypothesized that the effect of hypervolemia on syndecan-1 shedding, if any, might be negligible when compared with the effect of the inflammation in sepsis.

Previous studies showed that markers of glycocalyx shedding were rising during the ED stay [49, 70]. The level was generally decreasing during the ICU stay. However, rising level during ICU admission was associated with increased mortality [51]. In our study, we found an almost equal number of patients who had rising and falling levels in the ED and the pattern of changes was not associated with mortality. This suggested that the glycocalyx degradation, as measured by syndecan-1, did not uniformly increased during the ED stay. Furthermore, it was unclear that the change was caused by different degrees of shedding or impairment of the syndecan-1 clearance. The syndecan-1 level was associated with kidney function at baseline [73]. Decreased renal clearance of syndecan-1 was associated with increased plasma level [74]. Further study should investigate what and how sepsis treatment affects the level of glycocalyx shedding during the ED and ICU stay, especially considering the influence of the kidney function.

To our knowledge, this study is the first randomized trial that compared the different fluid rate strategies in the very early phase of resuscitation of septic patients in the emergency department. The type of resuscitation fluid was controlled provided that difference fluid type was associated with different magnitude of glycocalyx damages [44]. However, there are several notable limitations. First, the investigators, healthcare providers, and patients were not blinded to the study procedures, so potential biases may have affected in recognition and treatment in open-label trials. However, we measured objective outcomes that are less susceptible to misclassification. Second, despite of the appropriate randomization method, the participants between the groups still had different baseline characteristics. The adjusted analysis was performed to mitigate this disparity. Third, the assessment of glycocalyx integrity with direct visualization (e.g. intravital microscopy), or using mass spectrometry, potentially yields more accurate results than the detection of plasma syndecan-1 by the ELISA methods. However, measuring the syndecan-1 by is much more practical in clinical use. Syndecan-1 had negative correlation with the changes in glycocalyx thickness and positive correlation with changes in microvascular permeability [52]. Furthermore, Syndecan-1 was extensively studied regarding correlations with clinical outcome [75]. Higher level of syndecan-1 was associated with organ failures and mortality in patients with sepsis [49, 51].

Regarding the post-hoc analysis, this is the first study comparing the clinical significance of syndecan-1 levels at different time points in the early phase in the ED and their predictive ability on mortality. Our study is also one of a few ED-based studies that explore the relationship of syndecan-1 levels with fluid requirements and clinical outcomes. However, as this is an observational study, the analysis could be complicated by unmeasured confounders and selection bias. The estimation of the effect size might not be precise since we included a small number of patients. Moreover, as we included only patients with sepsis-induced hypoperfusion in this study, it is unclear whether the analysis of well-perfused septic patients will result similarly. We estimated the predictive ability of the syndecan-1 level to the mortality in this cohort. In order to prove generalizability, this needs validation in another testing cohort. Finally, different

studies that employed different assays showed markedly variable levels of the syndecan-1, limiting the comparability of each study [76].

This study implicated that administering limited rate of fluid in sepsis resuscitation in the emergency department did not increase harm when compared to the standard rate of resuscitation. According to the beneficial effect of limited fluid resuscitation in previous literature, studies with larger sample size are currently conducted, and would provide more conclusive results of patient-related outcomes [63, 77]

Conclusions

In conclusion, in patients with sepsis-induced hypoperfusion, administration of resuscitative fluid with limited fluid infusion rate resulted in similar magnitude of syndecan-1 changes and significantly reduced the volume used in the early resuscitation phase with compared with those resuscitated with standard rate approach. Larger studies with more participants are needed to improve the detection of difference in the degree of glycocalyx shedding and highlight the effects of different fluid strategies on the important clinical outcomes. Moreover, in the emergency department, syndecan-1 levels were associated with fluid requirement, sepsis severity and clinical outcomes. Syndecan-1 modestly predict sepsis mortality and could be useful in risk stratification of sepsis.

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VITA

NAME Jutamas Saoraya

INSTITUTIONS ATTENDED Chulalongkorn University

PUBLICATION

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