# Does Early Initiation of Renal Replacement Therapy Have an Impact on 28-day mortality in Critically Ill Patients with Acute Kidney Injury with Positive Furosemide Stress Test?: a Multicenter Randomized Controlled Trial

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จุฬาลงกรณ์มหาวิทยาลัย
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# การศึกษาทางคลินิกแบบสุ่มตัวอย่างหลายสถาบันว่าการเริ่มบำบัดทดแทนไตตั้งแต่ระยะแรกในผู้ป่วยวิกฤตที่มีภาวะไตวายเฉียบพลันในผู้ป่วยที่การทดสอบการตอบสนองต่อยาขับปัสสาวะฟูโรซีไมด์เป็นบวกมีผลต่ออัตราการเสียชีวิตที่ 28 วันหรือไม่

น.ส.ณัฏฐา ล้ำเลิศกุล

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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| Thesis Title | Does Early Initiation of Renal Replacement Therapy Have an Impact on 28-day mortality in Critically Ill Patients with Acute Kidney Injury with Positive Furosemide Stress Test?: a Multicenter Randomized Controlled Trial |
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ณัฏฐา ล้ำเลิศกุล : การศึกษาทางคลินิกแบบสุ่มตัวอย่างหลายสถาบันว่าการเริ่มบำบัดทดแทนไตตั้งแต่ระยะแรกในผู้ป่วยวิกฤตที่มีภาวะไตวายเฉียบพลันในผู้ป่วยที่การทดสอบการตอบสนองต่อยาขับปัสสาวะฟูโรซีไมด์เป็นบวกมีผลต่ออัตราการเสียชีวิตที่ 28 วันหรือไม่. ( Does Early Initiation of Renal Replacement Therapy Have an Impact on 28-day mortality in Critically Ill Patients with Acute Kidney Injury with Positive Furosemide Stress Test?: a Multicenter Randomized Controlled Trial) อ.ที่ปรึกษาหลัก : รศ. นพ.ณัฐชัย ศรีสวัสดิ์, อ.ที่ปรึกษาร่วม : รศ. นพ.ขจร ตีรณธนากุล

บทนำ: เวลาในการเริ่มการบำบัดทดแทนไตในผู้ป่วยที่มีภาวะไตวายเฉียบพลันรุนแรงยังไม่เป็นที่ทราบแน่ชัด พบว่าการตอบสนองต่อยาขับปัสสาวะฟูโรซีไมด์ช่วยทำนายโอกาสในการบำบัดทดแทนไตได้ดี ดังนั้น จึงเป็นที่มาของการศึกษาในการนำการทดสอบนี้เพื่อแยกผู้ป่วยที่มีโอกาสในการบำบัดทดแทนไตสูงและต่ำ เพื่อใช้เป็นแนวทางใหม่ในการศึกษาเกี่ยวกับระยะเวลาในการเริ่มบำบัดทดแทนไตให้มีประสิทธิภาพยิ่งขึ้น วิธีการ: การศึกษานี้เป็นแบบสุ่มตัวอย่างหลายสถาบันในหอผู้ป่วยวิกฤตเพื่อคัดกรองผู้ป่วยไตวายเฉียบพลันที่มีความเสี่ยงสูงต่อการบำบัดทดแทนไตและไม่ตอบสนองต่อยาขับปัสสาวะฟูโรซีไมด์เพื่อสุ่มตัวอย่างในการเริ่มบำบัดทดแทนไตเร็วหรือตามข้อบ่งชี้ ผลลัพธ์ที่ต้องการศึกษาคือ ความแตกต่างของอัตราการเสียชีวิตระหว่างการบำบัดทดแทนไตเร็วกับตามข้อบ่งชี้ที่ 28 วัน ผลการศึกษา: จากการให้ยาขับปัสสาวะฟูโรซีไมด์ในผู้ป่วยไตวายเฉียบพลันทั้งหมด 162 ราย มีผู้ป่วยที่ตอบสนองต่อยาฟูโรซีไมด์ 44 ราย และไม่ตอบสนองจำนวน 118 ราย ผู้ป่วยที่ตอบสนองต่อยาฟูโรซีไมด์ร้อยละ 13.6 มีความจำเป็นต้องได้รับการบำบัดทดแทนไต ในกลุ่มผู้ป่วยที่ไม่ตอบสนองต่อยาฟูโรซีไมด์ ผู้ป่วยร้อยละ 98.3 ในกลุ่มบำบัดทดแทนไตเร็ว และร้อยละ 75 ในกลุ่มบำบัดทดแทนไตตามข้อบ่งชี้ที่ได้รับการบำบัดทดแทนไต ไม่พบความแตกต่างระหว่างอัตราการเสียชีวิตที่ 28 วันของทั้งสองกลุ่ม (ร้อยละ 62.1 กับร้อยละ 58.3, p = 0.68) และสมดุลน้ำที่ 7 วัน หรืออัตราการฟอกไตที่ 28 วัน สรุป การทดสอบการตอบสนองต่อยาขับปัสสาวะฟูโรซีไมด์สามารถนำมาใช้แยกผู้ป่วยที่มีความเสี่ยงสูงและต่ำในการเริ่มบำบัดทดแทนไตได้ดี จากผลการศึกษายังไม่พบความแตกต่างระหว่างการเริ่มบำบัดทดแทนไตเร็วและตามข้อบ่งชี้

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สาขาวิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORD: Acute kidney injury, Renal replacement therapy, Furosemide stress test

Nuttha Lumlertgul : Does Early Initiation of Renal Replacement Therapy Have an Impact on 28-day mortality in Critically Ill Patients with Acute Kidney Injury with Positive Furosemide Stress Test?: a Multicenter Randomized Controlled Trial. Advisor: Assoc. Prof. Nattachai Srisawat Co-advisor: Assoc. Prof. KHAJOHN TIRANATHANAGUL

Background: The timing of initiation of renal replacement therapy (RRT) in severe acute kidney injury (AKI) remains controversial, with early initiation resulting in unnecessary therapy for some patients while expectant therapy may delay RRT for other patients. The furosemide stress test (FST) has been shown to predict the need for RRT and therefore could be used to exclude low-risk patients from enrollment in trials of RRT timing. Methods: FST was performed using intravenous furosemide (1 mg/kg in furosemide-naive patients or 1.5 mg/kg in previous furosemide users). FST-nonresponsive patients (urine output less than 200 mL in 2 h) were then randomized to early (initiation within 6 h) or standard (initiation by urgent indication) RRT. The primary outcome is 28-day difference in mortality rates between early and standard RRT. Results: FST was completed in 162 patients. Only 6/44 (13.6%) FST-responsive patients ultimately received RRT. Among 118 FST-nonresponsive patients, 98.3% in the early RRT arm and 75% in the standard RRT arm received RRT. We observed no differences in 28-day mortality (62.1 versus 58.3%, p = 0.68), 7-day fluid balance, or RRT dependence at day 28. Conclusion: The furosemide stress test appears to be feasible and effective in identifying patients for randomization to different RRT initiation times. There was no difference between 28-day mortality rates between furosemide-nonresponsive patients who were randomized to early or standard RRT initiation.

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# CHAPTER I: BACKGROUND AND RATIONALE

## Background

Acute kidney injury (AKI) is a common complication in intensive care units (ICU) and leads to increased short-term and long-term morbidity and mortality.[1] When to initiate renal replacement therapy (RRT) in acute kidney injury is controversial. Clinical symptoms and conventional markers i.e. blood urea nitrogen and creatinine lack accuracy for prediction of initiation of renal replacement therapy. Therefore, novel biomarkers maybe useful in guiding initiation of renal replacement therapy. Furosemide is a loop diuretic which can be used to assess intact glomerular filtration and renal tubular function. Furosemide stress test has recently been validated and demonstrated valuable for prediction of progression to severe acute kidney injury, renal replacement therapy, and death.[2, 3] In this study, we aim to use furosemide stress test in guiding the decision for RRT initiation by randomization of patients with furosemide stress test non-responsiveness to early or standard renal replacement therapy. The primary outcome is 28-day mortality.

## Research question

### Primary research question

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- Does early initiation of renal replacement therapy have an impact on 28-day mortality in critically ill patients with acute kidney injury with furosemide stress test non-responsiveness?

### Secondary research question

- Does early initiation of renal replacement therapy have an impact on renal recovery, 7-day fluid balance, RRT-free days, mechanical ventilator-free days, ICU-free days, ICU length of stay, hospital length of stay, dialysis dependence, and adverse events in critically ill patients with acute kidney injury with furosemide stress test non-responsiveness?

- Does early initiation of renal replacement therapy have an impact on changes in plasma neutrophil-gelatinase associated lipocalin (NGAL), N-terminal pro b-type natriuretic peptide (NT-proBNP), and angiopoietin-2 in critically ill patients with acute kidney injury with furosemide stress test non-responsiveness?
- Can furosemide stress test be used to identify acute kidney injury patients likely to receive renal replacement therapy in critically ill settings?

**Objectives**

1. To compare early and standard initiation of RRT in furosemide stress test-nonresponsive AKI patients for 28-day mortality
2. To compare early and standard initiation of RRT in furosemide stress test-nonresponsive AKI patients for renal recovery, 7-day fluid balance, RRT-free days, mechanical ventilator-free days, ICU-free days, ICU length of stay, hospital length of stay, dialysis dependence, and adverse events
3. To compare early and standard initiation of RRT in furosemide stress test-nonresponsive AKI patients for changes in plasma NGAL, serum NT-proBNP, and angiopoietin-2 during baseline, day 3, and day 7
4. To determine whether furosemide stress test could be used in a clinical trial setting to stratify AKI patients and determine the feasibility of using FST in this setting.

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**Hypothesis**

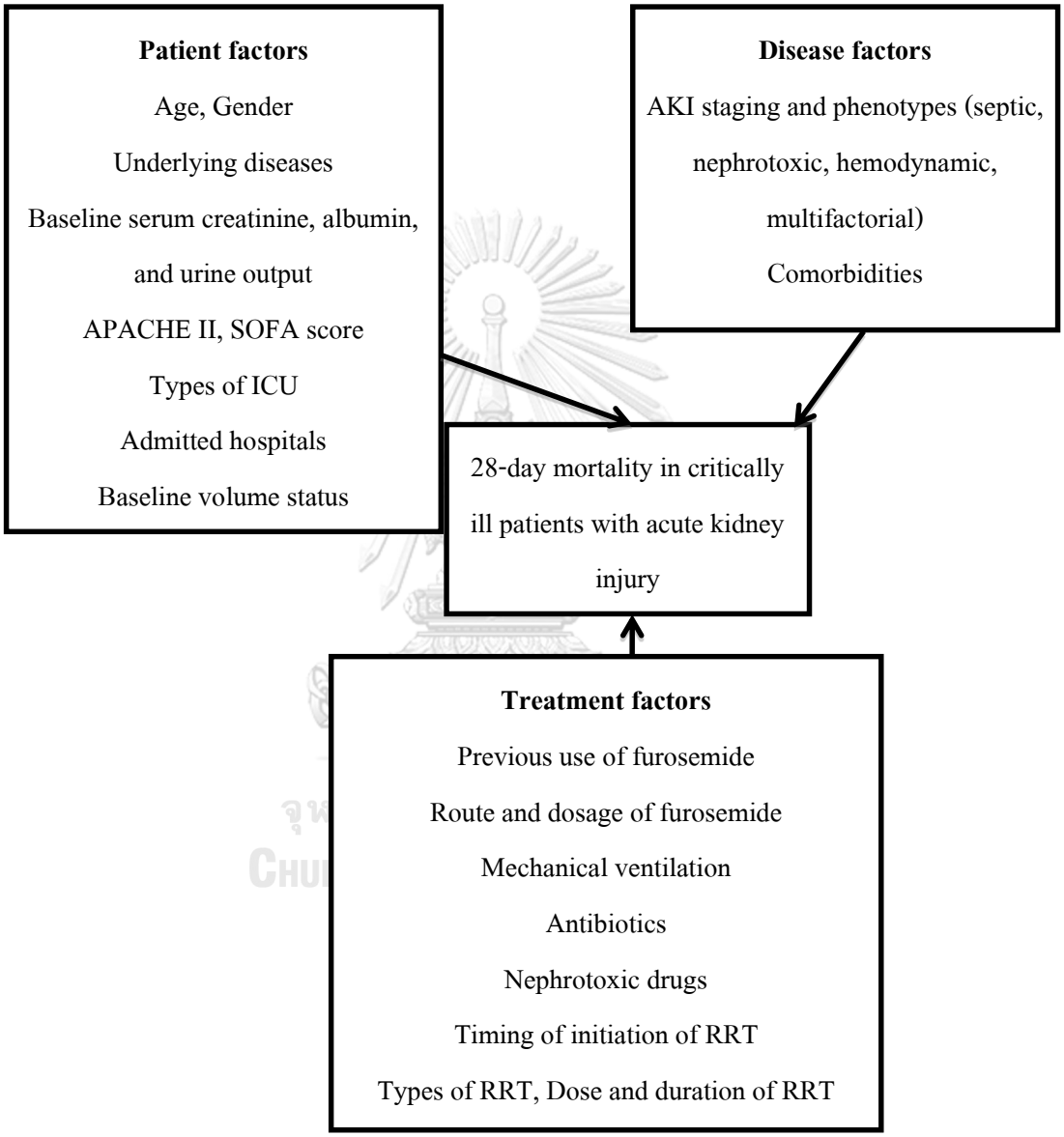
Early and standard initiation of RRT in furosemide stress test-nonresponsive AKI did not result in different 28-day mortality

**Research design**

This study is a therapeutic experimental multicentered, open-label, prospective, randomized controlled trial comparing early and standard initiation of RRT in critically AKI patients with furosemide stress test non-responsiveness.

**Conceptual framework**

*Figure 1* Conceptual framework of factors affecting 28-day mortality in critically ill patients with acute kidney injury



# CHAPTER II: REVIEW OF LITERATURE

## Introduction

Acute kidney injury (AKI) is a syndrome which kidney function acutely declines within hours or days. AKI is defined as an increase in serum creatinine at least 0.3 mg/dL from baseline within 48 hours, an increase at least 1.5 times of baseline creatinine, or a decrease of urine output less than 0.5 mL/kg/hour for at least 6 hours.[4] AKI staging is shown in table 1. AKI can lead to detrimental complications including dysregulation of fluid and electrolyte balance, increased inflammation, and uremic symptoms.[5] The largest multinational epidemiology study (AKI-EPI study) reports 57% incidence of AKI and 27% short-term mortality. The mortality rate increases with AKI staging.[1]

*Table 1* AKI staging by Kidney Disease Initiatives; Global outcomes 2012

| Stage | Creatinine | Urine output |
|---|---|---|
| 1 | 1.5 – 1.9 times baseline<br>OR<br>>= 0.3 mg/dL increase | < 0.5 mL/kg/h for 6-12 hours |
| 2 | 2.0 – 2.9 times baseline | < 0.5 mL/kg/h for >= 12 hours |
| 3 | 3.0 times baseline<br>OR<br>Increase in serum creatinine to >=4.0 mg/dL<br>OR<br>Initiation of renal replacement therapy<br>OR,<br>In patients < 18 years, decrease in eGFR to < 35 mL/min per 1.73 $m^2$ | < 0.3 mL/kg/h for >= 24 hours<br>OR<br>Anuria for >= 12 hours |

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In patients with severe acute kidney injury, renal replacement therapy (RRT) is the main therapy for renal support and treatment of complications e.g. fluid overload, metabolic acidosis, hyperkalemia, uremia etc. Conventional indications for RRT include refractory fluid overload,

refractory metabolic acidosis, refractory hyperkalemia, and uremic symptoms such as uremic encephalopathy and uremic pericarditis. Relative indications are renal support in multiorgan failure, immunomodulation in sepsis, $CO_2$ removal in respiratory failure, etc. Modality of RRT in intensive care units are categorized as intermittent hemodialysis (IHD), slow-low efficiency dialysis (SLED), continuous renal replacement therapy (CRRT), and peritoneal dialysis (PD). However, even after 50 years of RRT invention and widespread use, the mortality of patients who received RRT is still high. Therefore, timing of RRT initiation is considered a contributing factor for patients' mortality.

**Factors affecting decision to start RRT**

There are several factors that influence physicians regarding when to start RRT. Most importantly, benefits must be weighed against risks of RRT initiation. The severity of AKI must be considered in terms of creatinine and urea trajectories, urine output, fluid status, electrolyte derangement, acid base status, and complications of uremia. Severity of illness and the patients' capacity to cope with renal failure is another crucial factor. The severity of insult leading to AKI, non-renal organ dysfunction, pre-existing comorbidities, and potential recovery may trigger earlier initiation of RRT to unload high demand in patients with low capacity. However, potential risks of RRT must be taken into account including complications of line insertion, hemodynamic instability, and clearance of nutrients or drugs. Finally, physicians must consider environmental factors such as availability of machines and staff, patients' or relatives' wishes, and long-term prognosis.

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**Early vs. standard RRT initiation**

Early RRT may help physicians achieve better fluid control and early correction of acid-base and electrolyte derangement. In some patients with multi-organ failure, early RRT may help in terms of extracorporeal organ support and immunomodulation. However, early RRT may bring upon complications from too-early exposure to extracorporeal circuit such as maladaptive neuro-hormonal adaptation to RRT, iatrogenic hemodynamic insults, and membrane-induced inflammation. These can lead to impaired renal recovery and long-term dialysis dependence. Moreover, there may be increased risk for catheter-related complications. Standard RRT is a valid

option to avoid RRT-related complications. However, too late RRT initiation may risk patients to complications from uremia and fluid overload. Therefore, a question of early or standard RRT initiation has been debated for decades and there have been several studies aiming to clarify this question.

**Timing of RRT initiation**

Timing of RRT initiation is defined by various criteria; for example, blood urea nitrogen level, creatinine level, time from ICU admission to RRT initiation, time from AKI diagnosis to RRT initiation, AKI staging, or severity score. Initial studies used levels of blood urea nitrogen (BUN) and creatinine (Cr) to divide patients into "early" and "standard" RRT groups. Nevertheless, several factors can affect levels of these conventional markers including hydration status, muscle mass, steroid use, protein intake, and decrease Cr production during sepsis. In Program to Improve Care in Acute Renal Disease (PICARD) multicenter cohort study, patients were divided by BUN median value of 76 mg/dL into two groups. Patients with BUN higher than 76 mg/dL were associated with increased mortality.[6] Other observational studies which had used BUN for early and standard RRT groups had found conflicting results.[7, 8] Later, two randomized controlled trials by Bouman et al. and Jamale et al. also used BUN levels (< 45 and > 85 mg/dL in the first study and < 71 amd 101 mg/dL in the latter) to random patients for early or standard RRT.[9, 10] Both studies yielded negative results. Thus, BUN is not considered a useful tool for RRT initiation.

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Post-hoc studies of th(ATN trial) NIH Acute Renal Failure Trial Network Study/e VA and the Randomized Evaluation of Normal versus Augmented Level Replacement Therapy (RENAL tria1) used timing from ICU admission for early and standard RRT groups, which resulted in 6.7 days and 2.1 days, respectively. However, when reanalyzed by timing from AKI diagnosis to RRT initiation, the timing was comparable in both studies. Both studies also yielded negative results.[11, 12]

Later observational studies and randomized controlled had used AKI staging for RRT initiation as summarized in table 2 and 3. Recent meta-analyses had shown that early RRT might decrease mortality and control fluid-balance better than standard RRT.[13, 14] However, there was tremendous heterogeneity in patients' population, AKI diagnosis criteria, RRT modality, and

definition of early and standard RRT. Moreover, most studies were small-sized and single-center. Consequently, the results from the meta-analyses may be less applicable.

*Table 2* Observational studies comparing mortality rates between early and standard RRT

| Author | Year | Modality of RRT | No. | Early | Standard | Mortality |
|---|---|---|---|---|---|---|
| Retrospective | | | | | | |
| Elahi[15] | 2004 | CRRT | 64 | UO < 100 mL/h | Urea >= 30 mmol/L | 43% vs. 22%* |
| Liu[6] | 2006 | HD and CRRT | 243 | BUN < 76 mg/dL | BUN > 76 mg/dL | 61% vs. 80%* |
| Piccinni[16] | 2006 | CRRT | 80 | Sepsis < 12 h in ICU | Sepsis traditional | 54% vs. 61%* |
| Payen[17] | 2009 | HD and CRRT | 278 | <48 h in ICU | >48 h in ICU | 45% vs. 65%* |
| Bagshaw[18] | 2009 | HD and CRRT | 1,238 | Urea <= 24.2 mmol/L | Urea > 24.2 mmol/L | 53% vs. 71%* |
| Shiao[19] | 2009 | CRRT and HD | 98 | O/R | I/F | 43% vs. 75%* |
| Iyem[20] | 2009 | CRRT | 185 | UO <0.5 mL/kg/h and an increase in urea and cr 50% | UO >= 0.5 mL/kg/h and an increase in urea cr 50% in 48 h | 5% vs. 7% |
| Carl[8] | 2010 | CRRT | 147 | BUN < 66 mg/dL | BUN > 100 mg/dL | 52% vs. 68% |
| Ji[21] | 2011 | CRRT | 58 | UO < 100 mL/h <12 h | UO < 100 mL/h > 12 h | 9% vs. 38%* |
| Chou[22] | 2011 | CRRT and SLED | 370 | O/R | I/F | 71% vs. 70% |

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| Author | Year | Modality of RRT | No. | Early | Standard | Mortality |
|---|---|---|---|---|---|---|
| De Nascimento [7] | 2012 | PD and HD | 86 | BUN < 75 mg/dL | BUN > 75 mg/dL | 39% vs. 69%* |
| Oh[23] | 2012 | CRRT | 210 | <2 days[a] | >2 days[a] | 66% vs. 86%* |
| Wu[24] | 2012 | CRRT | 71 | R | I/F | 50% vs. 85%* |
| Leite[25] | 2013 | HD and SLED | 150 | F < 24 hours[b] | F > 24 hours[b] | 52% vs. 78%* |
| Shum[26] | 2013 | CRRT | 120 | R | F | 48% vs. 48% |
| Jun[27] | 2014 | CRRT | 439 | <7.1 hours[b] | >46 hours[b] | 36% vs. 40% |
| Prospective | | | | | | |
| Lim[28] | 2015 | CRRT | 140 | R/I | traditional | 50% vs. 34% |
| Crescenzi[29] | 2015 | CRRT | 1,658 | UO < 0.5 mL/kg/h < 6 h | Persistent (>12 h) oliguria | 60.9% vs. 76.9% |

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**Abbreviations:** PD; peritoneal dialysis, HD; hemodialysis, CRRT; continuous renal replacement therapy, BUN; blood urea nitrogen, RIFLE grading of acute kidney injury (O; none, R; risk, I; injury; F; failure), Cr; creatinine, UO; urine output, ICU; intensive care unit, a = days from starting pressors to starting CRRT, b = Time from RIFLE stage I or F to starting CRRT, vs.; versus *P < 0.05 vs. early start

*Table 3* Randomized controlled studies comparing the mortality rate between early and standard RRT

| Author | Year | Modality of RRT | No. | Early | Standard | Mortality |
|---|---|---|---|---|---|---|
| Bouman[9] | 2002 | CRRT | 71 | BUN 45 mg/dL | BUN 85 mg/dL | 31% vs. 25% |
| Durmaz[30] | 2003 | HD | 44 | Prophylactic | Traditional | 4% vs. 30%* |
| Sugahara[31] | 2004 | CRRT | 28 | UO <30 mL/h | UO <20 mL/h | 14% vs. 86%* |
| Jamale[10] | 2014 | HD | 248 | BUN 71 mg/dL | BUN 101 mg/dL | 21% vs. 12% |
| Wald[32] | 2015 | CRRT | 101 | < 12 h from AKI stage 2 | Traditional | 33% vs. 37% |

**Abbreviation:** HD; hemodialysis, CRRT; continuous renal replacement therapy, BUN; blood urea nitrogen, UO; urine output, AKI; acute kidney injury, vs.; versus

*P < 0.05 vs. early start

Recently, there have been three large randomized controlled trials regarding timing of RRT initiation; Artificial Kidney Initiation in Kidney Injury (AKIKI), Effect of Early vs Delayed Initiation of Renal Replacement Therapy on Mortality in Critically Ill Patients with Acute Kidney Injury (ELAIN), and Initiation of Dialysis Early Versus delayed in Intensive Care Unit (IDEAL-ICU) as shown in Table 4.[33-35]

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*Table 4* Comparison between AKIKI, ELAIN, and IDEAL-ICU trials

| Studies | Parameters | ELAIN[34] | AKIKI[35] | ICU-IDEAL[33] |
|---|---|---|---|---|
| Setting | | Single ICU, Germany Cardiac 47% | ICUs in 31 France Surgical 20% Medical 80% | ICUs in 24 France |
| Population | Inclusion criteria | KDIGO stage 2 for 12 hours | KDIGO stage 3 | failure – RIFLE stage |
| | | Plasma NGAL >150 ng/mL | | |
| | | Severe sepsis, Noradrenaline or adrenaline dose > 1.0 mcg/kg/min, refractory fluid overload, progression of nonrenal organ dysfunction (SOFA score >= (2 | Critically unwell mechanical or ) (vasopressors | 1st 48 hour of shock |
| | Exclusion criteria | eGFR < 30 mL/min | eGFR < 30 mL/min | Chronic RRT |
| Sample size | | 231 | 620 | 864 |
| Baseline characteristics | SOFA score (early vs. delayed) | 15.6 vs. 16 | 10.9 vs. 10.8 | 12.2 vs. 12.4 |

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| Studies | Parameters | ELAIN[34] | AKIKI[35] | IDEAL-ICU[33] |
|---|---|---|---|---|
| Intervention | Early RRT | Within 8 hours of stage 2 AKI | Within 6 hours of stage 3 AKI | Within 12 hours of meeting inclusion criteria |
| Control | Delayed RRT | Within 12hours of stage 3AKI, urine < 200 mL in 12 hours, BUN > 100 mg/dL, K > 6 mEq/L, organ edema with resistance to diuretics | BUN > 112 mg/dL, K > 6 mEq/L, pH < 15.7, acute pulmonary edema, oliguria/anuria > 72hours | 60-48hours post meeting inclusion criteria OR meeting emergency indications for RRT |
| | % of patients in delayed group that received RRT | 91%at a median of 25 hours post randomization | 51%at a median of 57 hours post randomization | 62% at a median of 51 hours post randomization |
| Modality of RRT | | CVVHDF100% | IHD 55%,CRRT 30% | IHD34%, CRRT 46%,Both20% |
| Primary outcome | Mortality | 90days | 60days | 90days |
| | Early versus delayed | %7.54 .vs %3.39 | 5.48vs. %7.49 | %54 .vs %58 |
| | p value | 03.0 | 79.0 | 0.38 |
| | Fragility index | 3 | 18 | 0 |
| Secondary outcomes | Duration of RRT, median days | 9vs. 25, = p 04.0 | NA | 4 vs. 2 days, p<0.001 |
| | Dialysis | At day90: %13 | At day60: %2 | 3% vs. 2%, p = |

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| | | | | |
|---|---|---|---|---|
| | dependence at 90 days | ,%15 .vsp = 8.0 | ,%5 .vsp = 12.0 | 1.00 |
| | Others | Early RRT; shorter mechanical ventilation and hospital length of stay | Early RRT; delayed diuresis, more catheter related-blood stream infections | Early RRT; fewer RRT-free days |

**Abbreviation** ELAIN, Effect of Early vs Delayed Initiation of Renal Replacement Therapy on Mortality in Critically Ill Patients With Acute Kidney Injury, AKIKI, Artificial Kidney Initiation in Kidney Injury; IDEAL-ICU, Initiation of Dialysis Early Versus delayed in Intensive Care Unit;RCT, randomized controlled trial; TBA, to be announced; RRT, renal replacement therapy;CRRT, continuous renal replacement therapy; IHD, intermittent hemodialysis; ICU, intensive care unit; CVVHDF, continuous venovenous hemodiafiltration, APACHE II, Acute Physiology and Chronic Health Evaluation Score; NGAL, neutrophil gelatinase associated lipocalin; SOFA, sequential organ failure assessment.

The ELAIN study is a single-center randomized controlled trial in Germany. The setting is 95% post-surgical patients. Early RRT group is patients with AKI stage 2, while standard RRT group includes patients who reached AKI stage 3. The modality of RRT is solely CRRT. There was significantly fewer 60-day mortality rates in early RRT group (39% vs. 55% ; $p$= 0.03). Moreover, patients in the early RRT group had shorter RRT days, mechanical ventilation days, and hospital length of stay.

The AKIKI study and IDEAL-ICU study are both multicenter randomized controlled trials. The main population is medical patients, specifically sepsis patients in IDEAL-ICU study. Early RRT group in both trials are patients with AKI stage 3, while standard RRT group are patients who reached conventional indications. The modality of RRT is upon the physicians' discretion. There were no differences in mortality rates between early and standard RRT group. Interestingly, 49% and 29% of patients in the standard group in the AKIKI and IDEAL-ICU studies spontaneously recovered from severe AKI and thus did not require RRT.

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These trials mainly differ in terms of population, definition of early and standard RRT group, and RRT modality. Therefore, a consensus cannot be reached whether physicians should start RRT early or not. However, there was a similar observation in the standard group from AKIKI and ELAIN trials that up to 50% patients could avoid RRT. In the AKIKI study, patients in the delayed group who never had RRT had significantly fewer mortality rates than those who started RRT due to emergency conditions (37% vs. 62%). For that reason, AKI staging may not be a suitable criteria for RRT initiation as there are some patients in AKI stage 3 who spontaneously recover and never require RRT. It is therefore vital to determine who are likely to receive RRT or not.

**Biomarkers and its utility for RRT initiation**

Various biomarkers have been discovered and validated for AKI prediction, AKI prognosis, and RRT requirement, for instance, cystatin C, urine neutrophil-gelatinase lipocalin (NGAL), plasma NGAL, urine tBinding -xIGF(2-TIMP) 2-issue Inhibitor Metalloproteinase .(7-IGFBP) 7-Protein A recent meta-analysis demonstrated a range of AUC-ROC of 0.72 to 0.86 in prediction of RRT initiation.[36] There has never been a study which utilizes these biomarkers for RRT initiation. A feasibility study by Srisawat et al. used plasma NGAL stratify patients into low-risk and high-risk groups. Patients with high at least 400 ng/mL were randomized to early and conventional RRT initiation. None of the patients in the low NGAL group required RRT. In the high NGAL group, early and standard RRT group did not have different mortality rates. However, 40% of patients in the standard RRT group required RRT. Thus, plasma NGAL has a high negative predictive value for excluding patients who are not likely to receive RRT, but its positive predictive value is rather low.[37]

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**Furosemide**

Furosemide is a loop diuretics. It has been widely used for diuresis in congestive heart failure and renal failure. After ingestion, furosemide is 50% absorbed enterally into circulation. After that, 90% of furosemide is bound to albumin and delivered to glomerulus. At glomerulus, furosemide is secreted via organic anion transporter at proximal tubule and secreted into tubular

lumen. Furosemide is then delivered by urine flow to thick ascending limb loop of Henle, inhibits Na-K-2Cl channel at medulla and cortex, and induces natriuresis and aquaresis.[38]

There are several factors affecting furosemide responsiveness at renal tubules. First, in patients with low serum albumin (< 2 g/dL), furosemide cannot be effectively brought to glomeruli and distributed in tissues. In proximal tubular cells, furosemide is converted from active form to inactive form by uridine diphosphate-glucuronyl transferase (UDTG). Albumin inhibits conversion from active to inactive forms. Therefore, low serum albumin can increase inactive forms of furosemide.[39] Second, albumin in urine binds with furosemide, making its free form less available. Albuminuria more than 4 g/L can bind with 50-75% of furosemide.[40, 41] Third, there is reduced excretion of inactive furosemide in patients with renal failure, making half-life of inactive furosemide longer than patients with normal renal function (2.8 vs. 1.5 hours). Moreover, in patients with GFR less than 15 ml/min, only 1/5 to 1/10 of furosemide is delivered to glomerulus.[42]

**Furosemide stress test**

Theoretically, furosemide responsiveness requires adequate glomerular filtration, intact proximal tubule, and thick ascending limb loop of Henle. It is therefore an ideal tool for testing renal function. Chawla et al. developed furosemide stress test and published in 2013. Seventy-seven adult patients in intensive care units with AKI stage 1 and 2 from acute tubular necrosis were included. (Acute tubular necrosis was diagnosed by George Washington Urinary Sediment Score >= 2), or fractional excretion of sodium (FeNa) >1.0%. Patients with baseline glomerular filtration rate < 30 ml/min/1.73 m2, hypovolemia, previous kidney transplant, pregnancy, obstructive uropathy, loop diuretics allergy, or previous RRT within 30 days were excluded. Then, they were administered 1 mg/kg of furosemide in naïve patients or 1.5 mg/kg of furosemide in previous furosemide use. Urine output less than 200 ml in 2 hours had an AUC-ROC of 0.87 for progression to AKI stage 3 with 0.87 sensitivity and 0.84 specificity.[2]

In 2015, Koyner et al. validated furosemide stress test along with other biomarkers including TIMP-2xIGFBP-7, plasma and urine NGAL, urine interleukin(IL)-18, kidney injury molecule (KIM)-1, uromodulin, urine Cr, and urine albumin and sodium. Furosemide stress test

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had an AUC-ROC of 0.86 for RRT initiation and 0.70 for death. It also outperformed other biomarkers for RRT prediction.[3]

In our study, we aim to utilize furosemide stress test non-responsiveness to select patients with high risk for RRT initiation and random to early and standard RRT initiation. Furosemide non-responsiveness is defined as urine output < 200 ml in 2 hours. The primary outcome is 28-day mortality. Secondary outcomes are renal recovery, 7-day fluid balance, RRT-free days, mechanical ventilator-free days, ICU-free days, ICU length of stay, hospital length of stay, dialysis dependence, adverse events.

**Exploratory endpoints**

For exploratory endpoints, we aim to compare changes in serum biomarkers between early and standard RRT groups at baseline, day 3, and day 7. Biomarkers of interests are plasma NGAL, serum NT-proBNP, and angiopoietin-2. Plasma NGAL is a kidney damage and inflammatory biomarker. It is associated with renal replacement therapy and mortality in AKI patients.[43] Serum NT-proBNP represents fluid status and its changes could represent different control in fluid balance.[44] Serum angiopoietin-2 is a circulating antagonistic ligand of the endothelial-specific Tie2 receptor and thus a potential marker of endothelial vascular permeability.[45] Furthermore, we aim to explore whether these biomarkers are associated with adverse outcomes e.g. mortality or renal non-recovery.

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# CHAPTER III: MATERIALS AND METHODS

**Population and sample**

- **Target population** AKI patients in intensive care units
- **Study population** AKI patients in medical and surgical intensive care units at 5 tertiary hospitals screened consecutively
- **Sample size**

Previous statistics has shown 66% mortality rate of AKI patients on RRT in intensive care units in King Chulalongkorn Memorial Hospital.

According to the ELAIN study, early RRT has 38. 4% mortality rate compared with 50.4% in standard RRT group (OR 0.61). The investigators use this data as reference as there are 50% of cardiac patients and we aim to use 100% CRRT in our study, so this would resemble the ELAIN study more than the others.[34]

Confidence interval level = 95%

$\alpha$ = 0.05 -> $Z\alpha_{/2}$ = 1.960

$\beta$ = 0.2 (power 80%) -> $Z\beta$ = 0.842

p1 = mortality rate in standard RRT group= 0.66

q1 = 1-p1 = 1-0.65 = 0.34

p2 = mortality rate in early RRT group = 0.40 (Odds ratio=0.61)

q2 = 1-p2 = 1-0.40 = 0.60

$r = n_{control}/n_{case} = 1$

p = (p1 + rp2)/(r + 1) = 0.53

q = 1- p = 1 – 0.525 = 0.49

$$n_1 = n_{case} = \frac{[z_{\alpha/2}\sqrt{(r+1)pq} + z_{\beta}\sqrt{r\,p_1q_1 + p_2q_2}\,]^2}{r\,(p_1 - p_2)^2}$$

**n = 58 for each group**

Total enrolled cases = 116 cases

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Moreover, we aim to use FST to risk stratify patients who would need RRT and not need RRT. At least **thirty** patients were required to detect a 50% absolute difference in the proportion of RRT between FST responders and FST nonresponders (standard group) with a power of 80% ($\beta$ = 0.2) at a 5% significance level ($\alpha$ = 0.05).

- **Participating centers** 5 hospitals

  King Chulalongkorn Memorial Hospital

  Nakornping Hospital

  Vajira Hospital

  Bhumibol Adulyadej Hospital

  Vajira Phuket Hospital

**Inclusion criteria**

1. All adult patients (>= 18 years old) Patients with AKI at any stage (defined by Kidney Disease Improving Global Outcomes (KDIGO) criteria)
2. Clinical diagnosis of acute tubular necrosis (e.g. presence of granular or epithelial cast, fractional excretion of sodium >= 1% , fractional excretion of urea >= 50% , plasma neutrophil gelatinase-associated lipocalin ( NGAL) >= 150 ng/ mL, absence of obstruction, glomerular or vascular disease)
3. Opinion of the treating team that the patient was well-resuscitated and euvolemic (e.g. fluid accumulation >= 5% , central venous pressure >= 8 mmHg, pulse pressure variation < 13% , inferior vena cava collapsibility index < 50% in spontaneously breathing patients or distensibility index < 18% in mechanically ventilated patients)
4. Opinion of the treating team that the patient had neither an emergent indication nor a contraindication to RRT.

**Exclusion criteria**

1. Baseline serum creatinine >= 2 (male) or >= 1.5 mg/dL (female)[11]
2. History of renal allograft
3. Known pregnancy
4. Allergy or known sensitivity to loop diuretics

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5. Moribund patients with expected death within 24 hours or whose survival to 28 days was unlikely due to an uncontrollable comorbidity ( i. e. end-stage liver or heart disease, untreatable malignancy)
6. Patients with advanced directives issued the desire not to be resuscitated
7. Prior treatment with RRT within 30 days
8. Serum albumin < 2 g/dL
9. Patients receiving extracorporeal membrane oxygenation or circulatory assistance

**Assumption**

Patients must be older than 18 years old and admitted in intensive care units. The volume status must be euvolemic. The cause of acute kidney injury must be from acute tubular necrosis and not from obstructive uropathy or hypovolemia.

**Keywords**

Acute kidney injury, furosemide stress test, renal replacement therapy

**Operational definition**

1. **Acute kidney injury (AKI)**

AKI is defined by Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury (2012) as an increase in serum creatinine at least 0.3 mg/dL from baseline within 48 hours, an increase at least 1.5 times of baseline creatinine, or a decrease of urine output less than 0.5 mL/kg/hour for at least 6 hours.[4] Baseline creatinine is defined as previous creatinine before hospital admission within 3 months. If there is no previous creatinine, use the lowest serum creatinine at hospital admission or calculate from Modification of Diet in Renal Disease (MDRD) formula; (175 x $SCr^{(-1.154)}$ x $Age^{(-0.203)}$ x 0.742 (if female) to eGFR 75 ml/min/1.73 $m^2$.

2. **Renal replacement therapy**

Renal replacement therapy is defined as continuous renal replacement therapy for the first 7 days after RRT inception. Vascular access is nontunneled catheter with at least 11.5 French diameter in internal jugular vein or femoral vein. Insertion of catheters should be done under ultrasound guidance with universal precaution. The modality of CRRT is continuous venovenous

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hemofiltration with integrated or separated system. Dialyzer should be changed every 72 hours or after clots. Blood flow rate should be at least 150 ml per minute. Delivered dialysis dose should be 20-25 ml/ kg/ h. Dialysate and replacement solutions can be adjusted to achieve normal electrolyte and acid-base. Anticoagulation can be regional citrate anticoagulation, heparin, or none depending on each patient's condition. During CRRT session, mean arterial pressure should be kept at least 65 mmHg. Ultrafiltration should be adjusted by nephrologists or primary physicians to achieve euvolemia.

**3. Timing of RRT initiation**

- Early RRT is defined as RRT initiation within 6 hours after nonresponsiveness to furosemide stress test
- Standard RRT is defined as RRT initiation by conventional indications

**4. Indications for standard RRT**

- Refractory severe acidosis defined as pH < 7.15, base deficit > 5 mEq/L, or $HCO_3^-$ < 12 mEq/L non-responsive to medications
- Refractory volume overload defined as severe hypoxemia ($PaO_2/FiO_2$ < 200) OR pulmonary edema from chest radiography non-responsive to diuretics
- Refractory hyperkalemia defined as serum [K+] >= 6 mmol/L or electrocardiographic changes from hyperkalemia non-responsive to medications
- Uremic signs or symptoms defined as uremic encephalopathy, uremic pericarditis or clinical signs and symptoms attributed to uremic toxin accumulation unexplained by other causes
- Serum BUN >= 100 mg/dL

**5. Furosemide stress test (FST)**

- Administration of furosemide 1.0 mg/kg in furosemide-naïve patients and 1.5 mg/kg in previous furosemide use within 7 days
- Body weight is determined from ideal body weight
- Furosemide stress test interpretation

  Responsive: Total urine output > 200 mL after 2 hours of FST

  Non-responsive: Total urine output <= 200 mL after 2hours FST

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6. **Fluid balance** is defined as total intake (intravenous fluids, feeding, blood components, medications, substitution fluids) minus output (urine + ultrafiltrate + others)
7. **Fluid accumulation** is defined as fluid balance (Litres) divided by body weight at first ICU admission (kg) x 100
8. **Fluid overload** is defined as percentage of fluid accumulation more than 10%
9. **Renal recovery** is defined by spontaneous urine output more than 1,000 mL per day or 2,000 mL per day with diuretics AND did not require RRT within 7 days according to AKIKI study.[35]
10. **Renal replacement therapy-free days** (censored at 28 days) is defined as days without RRT within 28 days after study enrollment
11. **Mechanical ventilator-free days** (censored at 28 days) is defined as days free from mechanical ventilation within 28 days after study enrollment
12. **Intensive care units-free days** (censored at 28 days) is defined as days that patients are out of ICU within 28 days after study enrollment
13. **Adverse events** is defined as follows:

- RRT-associated hemodynamic instability defined as hypotension requiring one of: initiation of a vasopressor during RRT session or need to escalate dose of a vasopressor during the RRT session or premature discontinuation of RRT session due to blood pressure drop or any other intervention to stabilize blood pressure during the dialysis session
- Arrhythmia, or seizure on RRT As noted in the medical chart
- Hypokalemia If serum potassium was below 3.0 mEq/L at any time during the study period
- Hypophosphatemia If serum phosphate was below 1.5 mg/dL at any time during the study period
- Hypocalcemia If albumin-adjusted total calcium was below 8 mg/dL at any time during the study period
- Hemorrhage at site of central venous catheter (CVC) insertion defined as bleeding at the puncture site requiring transfusion of >= 1 unit(s) of packed red blood cells within 12 hours following insertion and/or surgical intervention/repair

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- Pneumothorax ( for catheters placed in the internal jugular or subclavian positions) defined as air in the pleural space on routine chest x-ray that is performed following CVC insertion; further qualified by requirement for chest tube placement
- CVC-associated bacteremia defined as bloodstream infection in 2 blood culture sets (one drawn from dialysis catheter and the other from another site) with no proven alternative source for bloodstream infection as per ICU attending OR culture-positive recovery of the same organism from the dialysis CVC upon removal OR culture-positive from the dialysis catheter within 2 hours prior to positive hemoculture from peripheral sites
- Ultrasonographically confirmed thrombus attributed to CVC defined as any confirmed occlusive or non-occlusive thrombus in the vein in which a CVC was placed (or remains in place) or in the venous system drained by the vein in which the CVC was placed; further qualified by presence or absence of pulmonary embolism
- Air embolism or suspected air embolism As documented in the medical record
- Arterial puncture at CVC insertion As reported in the documentation of the CVC placement
- CVC malfunction Defined as inability to deliver blood flow for renal replacement therapy from malposition, occlusions by intraluminal thrombus or extrinsic fibrin sheath that require exchange or removal of the catheter.

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**Observation and measurement**

- Independent variable; time to initiate RRT ( early or standard) after FST non-responsiveness
- Dependent variables; 28-day mortality, renal recovery, 7-day fluid balance, RRT-free days, mechanical ventilator-free days, ICU-free days, ICU length of stay, hospital length of stay, dialysis dependence, and adverse events, and changes in plasma NGAL, serum NT-proBNP, and serum angiopoietin-2 from baseline to day 3 and day 7
- Control variables; RRT modality, dosage, anticoagulation

**Study methodology**

The trial was registered at clinicaltrials.gov (NCT02730117).

**Screening**

All adult patients (>= 18 years old) admitted to the ICU were screened. We considered the patients to be provisionally eligible if all the inclusion criteria were met, and no exclusion criteria were present. The investigators informed patients or their surrogates about the trial both orally and with a written document. Coinvestigators at each participating site were responsible for enrolling patients, ensuring adherence to the protocol, and completing the case record form.

Informed consent was obtained from participating patients or their substitute decision-makers before the FST was performed.

**Furosemide stress test**

FST was performed by giving intravenous furosemide 1 mg/kg to naive patients or 1.5 mg/kg to patients with a history of furosemide use within 7 days. Urine output was measured hourly and, if the urine output exceeded 200 mL for the subsequent 2 h, the patient was considered to be FST responsive. Patients with a urine output less than 200 mL in 2 h were considered FST nonresponsive and underwent randomization.

Blood pressure, heart rate, and urine output must be monitored hourly until 6 hours after FST. If there is hypovolemia or hypotension from furosemide administration, physicians can consider intravenous fluid to replace volume.

Record any adverse events or side effects after furosemide administration e. g. hypotension, hypokalemia, hypomagnesemia, etc.

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**Randomization**

We randomized patients 1: 1 to early or standard RRT initiation using a randomly permuted block of four, stratified by center and type of ICU.

**Renal replacement therapy**

Patients randomized to early RRT are to receive RRT within 6 h of randomization. The 6-h period is for the establishment of vascular access and RRT initiation.

In the standard RRT group, RRT is initiated only if one of the following criteria were met: blood urea nitrogen >= 100 mg/dL, serum potassium > 6 mmol/L, serum bicarbonate < 12

mmol/L or pH < 7.15, $PaO_2/FiO_2$ ratio < 200, or chest radiograph compatible with pulmonary edema.

In FST-responsive groups, patients will be monitored for indications of RRT by physicians until 72 hours. RRT is initiated by conventional criteria as in the standard RRT group.

**Blood sample collection and data collection**

In FST-nonresponsive patients, 10-mL blood is collected at baseline, day 3, and day 7 for measurement of plasma NGAL, NT-proBNP, and serum angiopoietin-2

Data is recorded on day 0 (study enrollment), 1,2,3,7,14,28 for vital signs, body weight, fluid balance, mechanical ventilation, vasopressors, laboratory investigations, RRT parameters, renal recovery, mortality, and adverse events

**RRT discontinuation criteria**

Physicians may consider stopping RRT if patients have renal recovery ( as defined above), RRT-related adverse events (severe hemodynamic instability unable to correct while on CRRT circuit), or patients or relatives express their wishes to withdraw from RRT.

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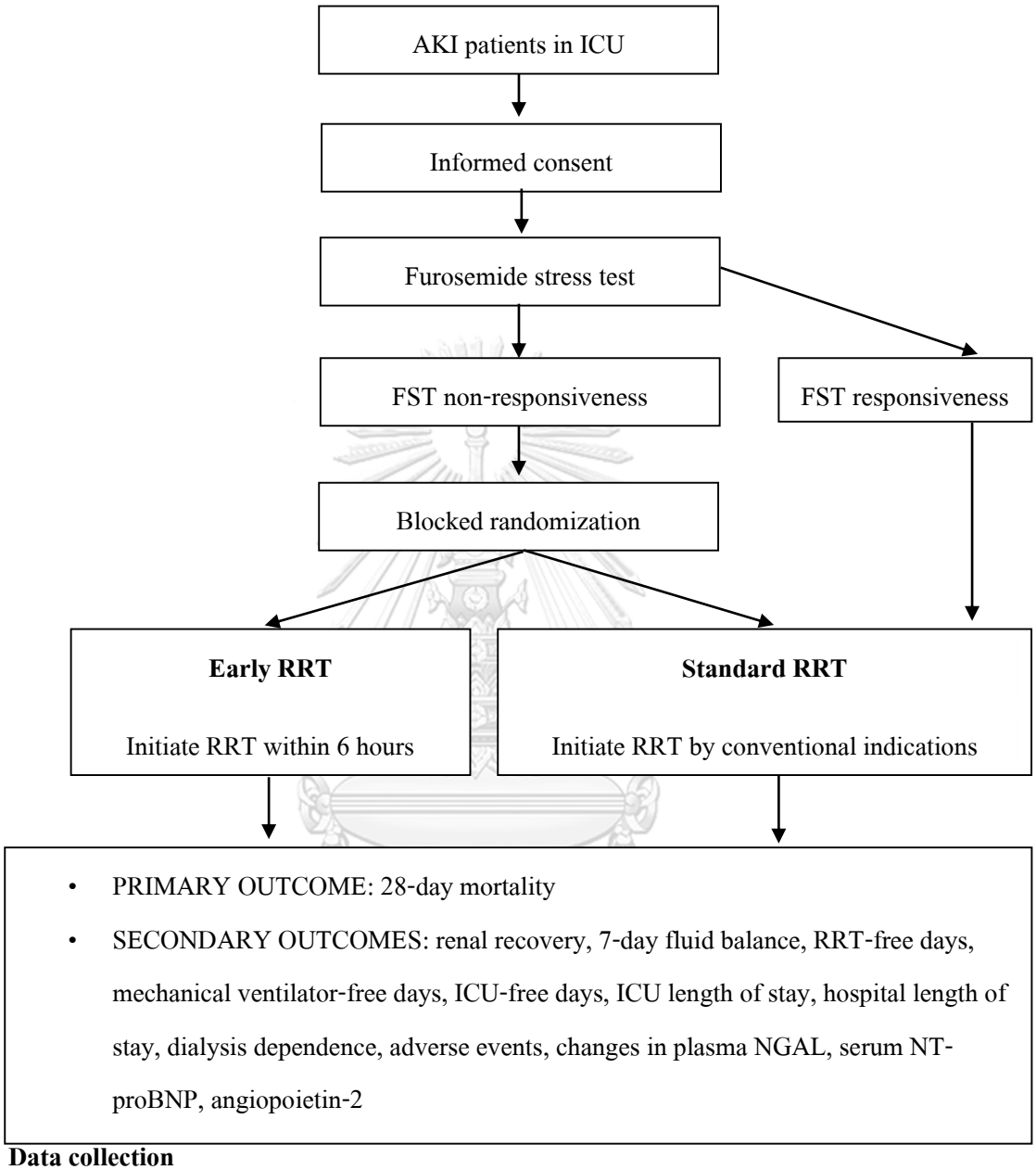
*Figure 2* Study flow




**Data collection**

Baseline characteristics, severity score, comorbidities, current medications, ICU admission date, hospital admission date, fluid accumulation, urine output, and laboratory investigations are recorded at Day 0. Then, vital signs and hemodynamic parameters (heart rate, blood pressure, central venous pressure), body weight, fluid balance, laboratory investigations, interventions (mechanical ventilation, vasopressors, fluid intake), fluid balance, RRT data are recorded at day 1,2,3,7,14,28. Adverse events are recorded by each site's primary investigator.

Any adverse event must be adjudicated by primary physician and primary investigator to determine if they are RRT-related adverse events or not.

*Table 5* Data collection

| Day | 0 | 1,2 | 3 | 7 | 14 | 28 | Data collection |
|---|---|---|---|---|---|---|---|
| Patient factors | | | | | | | |
| Inclusion and exclusion criteria | X | | | | | | Prospective recording |
| Demographic data and history | X | | | | | | |
| Types of ICU | X | | | | | | |
| APACHE II and SOFA | X | | | | | | |
| Hemodynamic data | X | X | X | X | X | X | |
| Underlying diseases | X | | | | | | |
| Disease factors | | | | | | | |
| AKI staging and etiology | X | | | | | | |
| Treatment factors | | | | | | | |
| Mechanical ventilation | X | X | X | X | X | X | |
| Vasopressors | X | X | X | X | X | X | |
| Timing, dose, duration, anticoagulant | X | X | | | | | |
| Nephrotoxic agents | X | X | X | X | X | X | |
| Outcome variables | | | | | | | |
| Urine output | X | X | X | X | X | X | |
| Laboratory tests | X | X | X | X | X | X | |
| Fluid balance | X | X | X | X | | | |
| Dead or alive status | X | X | X | X | X | X | |
| Mechanical ventilator-free days, ICU-free days, RRT-free days | | | | | | X | |
| Body weight, Fluid accumulation | X | X | X | X | | | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hospital length of stay,<br>ICU length of stay | | | | | | X | |
| Plasma NGAL | X | | X | X | | | |
| proBNP-Serum NT | X | | X | X | | | |
| 2-Serum angiopoietin | X | | X | X | | | |
| Adverse events | X | X | X | X | X | X | |

**Data analysis**

All analyses adhered to the intention-to-treat principle. Categorical data are described as numbers and percentages and compared between treatment groups using Chi-square or Fisher's exact test. Continuous variables are described as means (with standard deviations (SD)) or medians (with interquartile range (IQR)) and compared between each group using unpaired t test in normally distributed data or Wilcoxon rank sum test for non-normal data. Overall survival for all patients was estimated by the Kaplan-Meier method. A log-rank test was used to compare time to death between treatment arms and secondarily among patients undergoing RRT versus no RRT, and for patients with positive versus negative FST. The univariate Cox proportional hazard regression model was used to determine factors associated with RRT requirement in the standard arms using p values < 0.10, and a multivariate model was analyzed using significant factors from the univariate model, including gender. Data from all the patients were censored at the time of death or at day 28. Severity score, laboratory data, and physiological data between days 0, 3, and 7 were computed using repeated measures analysis of variance (ANOVA) for differences within groups and generalized estimating equations for differences between groups. Significant levels are determined at 0.05. All analyses were performed using Stata 14.0.

Interim analyses will be done at 80 and 120 patients. If the difference in mortality exceeds 25% between groups or the difference in any RRT-related adverse events exceeds 5%, the investigators will stop the study as continuation may increase harms to participants.

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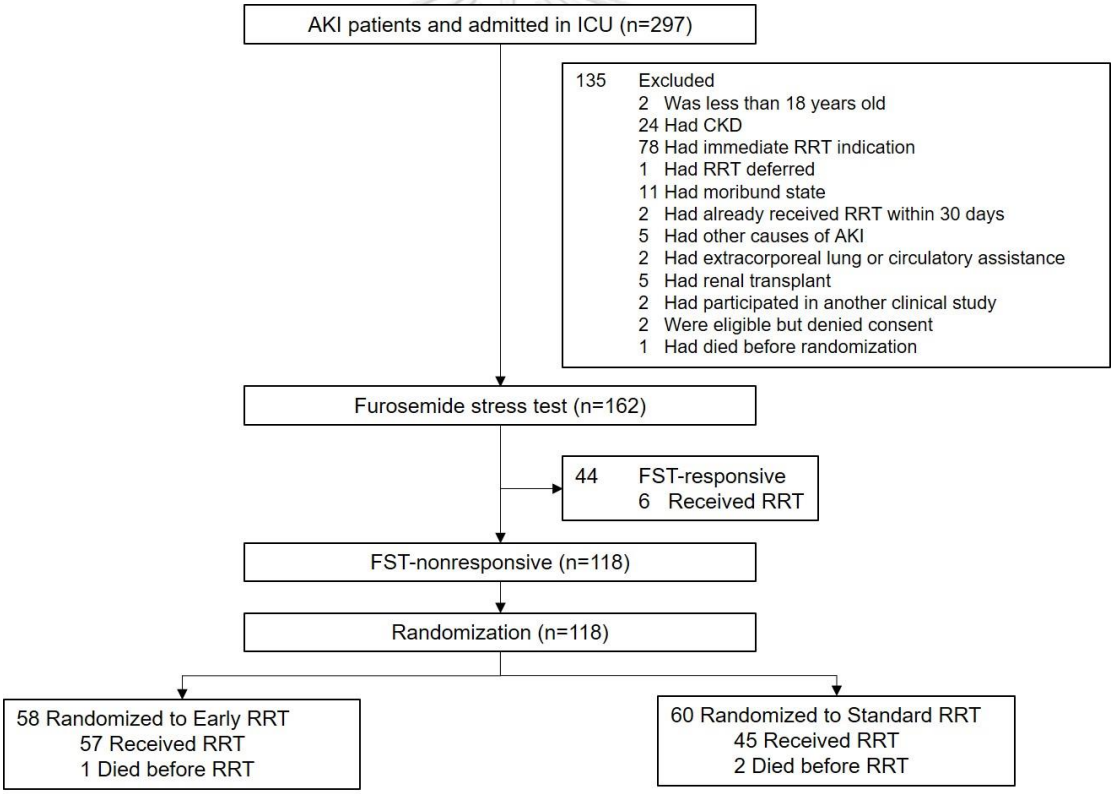
# CHAPTER IV: RESULTS

## Cohort characteristics and feasibility outcomes

Among 297 patients with AKI potentially eligible for inclusion in this trial, 162 patients underwent FST (Fig. 3). Informed consent was obtained before furosemide stress test. Forty-four patients were FST responsive, while 118 patients were FST-nonresponsive and were randomized to early RRT (n= 58) or standard RRT (n= 60). Of 58 patients in the early RRT group, 57 patients received RRT, while 1 patient died before RRT. Of 60 patients in the standard RRT group, 45 patients received RRT, while 2 patients died before RRT.

*Figure 3* Flow chart of patients' allocation




AKI was diagnosed in all patients before FST was performed. The diagnosis criteria was by creatinine criteria in 69/162 (42.6%) and by urine output criteria in 93/162 (57.4%). Acute tubular necrosis was diagnosed by urine casts, fractional excretion of sodium or urea, and plasma NGAL in 26.3%, 16.9%, and 56.8%, respectively. Compliance with the study protocol for all patients is shown in Table 6. Sites were able to perform FST in all eligible patients. The FST successfully excluded patients at low risk for RRT: 6/44 (13.6%) of FST-responsive patients

subsequently underwent RRT. Conversely, among FST-nonresponsive patients randomized to standard RRT, 45/60 (75%) underwent RRT ($p < 0.001$).

*Table 6* Study protocol compliance

| Parameters | FST-nonresponsive (n=118) | | FST-responsive (n=44) |
|---|---|---|---|
| | Early RRT (n = 58) | Standard RRT (n=60) | |
| FST completion, n (%) | 58 (100) | 60 (100) | 44 (100) |
| RRT, n (%) | 57 (98.3) | 45 (75) | 6 (13.6) |
| Initiation of RRT within 6 hours of randomization, n (%)[a] | 49/58 (84.5%) | N/A | N/A |
| Initiation of RRT within 12 hours of randomization, n (%)[b] | 55/58 (94.8) | N/A | N/A |
| Adherence to standard RRT initiation | N/A | 45/45 (100%) | 6/6 (100%) |
| Death after meeting RRT criteria but prior to RRT initiation, n (%) | 1 (1.7) | 2 (3.3) | 0 (0) |
| Loss to follow-up, n (%) | 0 (0) | 0 (0) | 0 (0) |

FST, furosemide stress test

[a] Early RRT – RRT initiation within 6 hours after randomization

Standard RRT – RRT initiation according to standard indications

[b] One patient died before RRT initiation. Two patients received RRT but later than 12 hours due to necessity for interventions.

Table 7 shows differences of demographic, clinical, and biochemical data between FST-responsive and FST-nonresponsive groups. Patients who were FST-nonresponsive had higher APACHE II score, SOFA score, AKI stage, and blood urea nitrogen levels. In addition, more patients in FST-nonresponsive group were in medical ICU and on vasopressors. Sepsis was present in 52.3% of FST-responsive patients compared with 58.5% of FST-nonresponsive patients. Of the 44 FST-responsive patients, 34.1% died, whereas 60.2% of the FST-nonresponsive patients died ($p = 0.003$).

*Table 7* Demographic, clinical, and biochemical data between FST- nonresponsive and FST-responsive patients

| Parameters | FST-nonresponsive (n = 118) | FST-responsive (n=44) | *p* value |
|---|---|---|---|
| Age, years, mean (SD) | 67.1 (15.8) | 61.6 (16.7) | 0.055 |
| Male, n (%) | 58 (49.2) | 29 (65.9) | 0.057 |
| ICU, n (%)<br>Medical<br>Surgical | <br>80 (67.8)<br>38 (32.2) | <br>22 (50)<br>22 (50) | **0.037** |
| Mechanical ventilation, n (%) | 98 (83.1) | 38 (86.4) | 0.61 |
| Vasopressors, n (%) | 92 (78) | 26 (59.1) | 0.016 |
| Sepsis, n (%) | 69 (58.5) | 23 (52.3) | 0.48 |
| APACHE II score, mean (SD) | 23.1 (6.7) | 19.0 (5.3) | **< 0.001** |
| SOFA score, mean (SD) | 12.0 (3.7) | 8.6 (3.6) | **< 0.001** |
| Non-renal SOFA score, mean (SD) | 9.5 (3.7) | 7.0 (3.5) | **< 0.001** |
| Baseline serum creatinine, mg/dL, mean (SD) | 1.08 (0.41) | 1.09 (0.36) | 0.93 |
| Estimated GFR, mL/min/1.73m$^2$, mean (SD)* | 70.1 (25.5) | 73.3 (27.4) | 0.49 |
| AKI staging, n (%)<br>1<br>2<br>3 | <br>23 (19.5)<br>43 (36.4)<br>52 (44.1) | <br>21 (47.7)<br>12 (27.3)<br>11 (25) | **0.001** |
| Blood Urea Nitrogen at enrollment, mg/dL, median [IQR] | 47.5 [33.75-66.25] | 37 [29-49] | **0.02** |
| Serum creatinine at enrollment, mg/dL, median [IQR] | 2 [2-3] | 2 [2-3] | 0.43 |
| RRT, n (%) | 103 (87.3) | 6 (13.6) | **< 0.001** |
| Mortality, n (%) | 71 (60.2) | 15 (34.1) | **0.003** |

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Data is reported by mean ± standard deviation unless indicated otherwise

*eGFR by CKD-EPI creatinine equation (2009)

ICU, intensive care unit; APACHE II, acute physiology and chronic health evaluation; SOFA, Sequential Organ Failure Assessment ; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury; RRT, renal replacement therapy

When visualized by receiver operating characteristics, FST had a higher area under the curve (AUC) (0.83) than APACHE II (0.71), SOFA (0.75), and nonrenal SOFA score (0.72) for the prediction of RRT. (Table 8)

*Table 8* Multivariable logistic regression on parameters to predict RRT

| Parameters | AUC | 95% CI |
|---|---|---|
| FST | 0.83 | 0.75 - 0.91 |
| APACHE II | 0.71 | 0.63 – 0.79 |
| SOFA | 0.75 | 0.67 – 0.84 |
| Non-renal SOFA | 0.72 | 0.63 – 0.80 |

FST, furosemide stress test; APACHE II, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment

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Randomization appeared to be successful since baseline characteristics were well balanced between treatment arms, except for APACHE II score. The early RRT group had a significantly higher APACHE II score compared with the standard RRT group (24.5 versus 21.8, $p = 0.027$). Most patients had AKI stage 2 and 3 (80.0%). Sepsis was present in 58.6% (Table 9).

*Table 9* Demographic, clinical, and biochemical data between early RRT and standard RRT patients

| Parameters | Early RRT (n = 58) | Standard RRT (n=60) |
|---|---|---|
| Age, years, mean (SD) | 67.5 (15.0) | 66.7 (16.7) |
| Male, n (%) | 29 (50) | 29 (48.3) |
| ICU, n(%)<br>Medical<br>Surgical | <br>40 (69)<br>18 (31) | <br>40 (66.7)<br>20 (33.3) |
| Mechanical ventilation, n (%) | 48 (82.8) | 50 (83.3) |
| Vasopressors, n (%) | 45 (77.6) | 47 (78.3) |
| Sepsis, n (%) | 37 (63.8) | 32 (53.3) |
| APACHE II score, mean (SD) | 24.5 (6.4) | 21.8 (6.9) |
| SOFA score, mean (SD) | 12.7 (3.3) | 11.4 (4.0) |
| Non-renal SOFA score, mean (SD) | 9.9 (3.3) | 9.1 (4.1) |
| Baseline serum creatinine, mg/dL, mean (SD) | 1.14 (0.44) | 1.03 (0.37) |
| Estimated GFR, mL/min/1.73m$^2$, mean (SD)* | 70.31 (28.1) | 69.98 (22.8) |
| AKI staging, n (%)<br>1<br>2<br>3 | <br>11 (19)<br>27 (46.6)<br>20 (34.5) | <br>12 (20)<br>16 (26.7)<br>32 (53.3) |
| Blood Urea Nitrogen at enrollment, mg/dL, median [IQR] | 42 [37-78] | 51 [37.5-61.25] |
| Serum creatinine at enrollment, mg/dL, median [IQR] | 2 [2-3] | 2.5 [2-3] |

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| Parameters | Early RRT (n = 58) | Standard RRT (n=60) |
|---|---|---|
| Co-morbidities, n (%) | | |
| Hypertension | 29 (50) | 24 (56.7) |
| Diabetes | 14 (24.1) | 15 (25) |
| Dyslipidemia | 16 (27.6) | 16 (26.7) |
| Ischemic heart disease | 12 (20.7) | 10 (16.7) |
| Malignancy | 12 (20.7) | 8 (13.3) |
| Cerebrovascular disease | 5 (8.6) | 7 (11.7) |
| Chronic liver disease | 10 (17.2) | 11 (18.3) |
| Nephrotoxic drugs, n (%) | | |
| Colistin | 5 (8.6) | 10 (16.7) |
| Vancomycin | 1 (1.7) | 1 (1.7) |
| Contrast | 8 (13.8) | 11 (18.3) |
| Aminoglycosides | 2 (3.4) | 2 (3.3) |
| Amphotericin | 2 (3.4) | 0 (0) |
| NSAIDs | 2 (3.4) | 1 (1.7) |
| Cardiac surgery, n (%) | 13 (22.4) | 8 (13.3) |
| Treatment limitation, n (%) | 12 (20.7) | 10 (16.7) |
| Fluid accumulation at randomization, mL, median [IQR] | 4763 [2837-8515] | 5114 [2050-8803] |
| Percentage of fluid overload, median [IQR][b] | 9.53 [3.43-19.68] | 7.63 [2.10-12.02] |
| Baseline NGAL, ng/mL, median [IQR] | 625 [376-1362] | 860 [447-1204] |
| Baseline NT-proBNP, pg/mL, median [IQR] | 4301 [515-35000] | 5844 [869-10007] |
| Baseline angiopoietin-2, ng/mL, median [IQR] | 16784 [8649-35545] | 22294 [12539-33186] |

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Data is reported by mean ± standard deviation unless indicated otherwise

*eGFR is calculated by CKD-EPI creatinine equation (2009)

[a] Treatment limitation is defined as withholding or withdrawal of patients from the treatment of primary disease either by the surrogates' decision after a period of intensive care management

[b] Fluid overload is calculated by total volume of fluid accumulation (intake – output) since ICU admission divided by body weight on admission and reported in percent.

ICU, intensive care unit; APACHE II, acute physiology and chronic health evaluation; SOFA, Sequential Organ Failure Assessment; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury; RRT, renal replacement therapy; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal prohormone of brain natriuretic peptide

Median time from randomization to RRT initiation was 2 (IQR 1–3) h in the early RRT group and 21 (IQR 17–49) h in the standard RRT group (difference = 19 h; $p < 0.001$). The median time from ICU admission to RRT initiation and median time from oliguria to RRT initiation in the early and standard RRT groups was 22 versus 100 h and 17 versus 38 h ($p < 0.001$) in both groups (Table 10). No patients were lost to follow-up for the survival status at day 28.

*Table 10* Duration parameters in intervention trial

| Parameters | Early RRT (n=58) | Standard RRT (n=60) | *p* value |
|---|---|---|---|
| Time from randomization to RRT, hours, median [IQR] | 2 [1-3] | 21 [16.75-48.5] | < 0.001 |
| Time from ICU admission to RRT, hours, median [IQR] | 22 [14-51] | 100 [25-257] | <0.001 |
| Time from oliguria to RRT, hours, median [IQR] | 17 [11-24] | 37.5 [30-55] | <0.001 |
| Fluid accumulation from randomization to RRT, mL, median [IQR] | 4763 [2837-8515] | 8659 [4388-10465] | 0.02 |

RRT, renal replacement therapy; ICU, intensive care unit

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**Primary outcomes**

In the early RRT arm, 57 out of 58 patients received RRT as 1 patient died before RRT initiation. In the standard RRT group, 45 out of 60 (75.0%) eventually met the prespecified indications and received RRT and 2 died prior to RRT. Interestingly, 15 out of 60 (25%) showed spontaneous renal recovery (Fig. 3). In the standard arm, multivariate Cox proportional hazard regression analysis showed that SOFA score, sepsis, and baseline plasma NGAL were significant predictors for RRT requirement. Patients who spontaneously recovered had median baseline plasma NGAL level of 518.5 (IQR 397.5–641.5) ng/mL compared with 885.5 (IQR 450–1320) ng/mL in those who eventually required RRT. Plasma NGAL had an adjusted hazard ratio (HR) of 1.06 (95% confidence interval (CI) 1.01–1.12; $p$ = 0.024) for RRT requirement. Cumulative fluid balance from ICU admission to randomization was comparable between both groups (4763 (IQR 2837–8515) mL in the early group versus 5114 (IQR 2050–8803) mL in the standard group). RRT prescription including CVVH dose and median ultrafiltration rate per day did not differ between both groups.

Mortality rates were estimated by the Kaplan-Meier method. The overall mortality at day 28 was 60.2%. The 28-day mortality rate by intention-to-treat analysis in the early RRT group did not differ from the standard RRT group (62.1% versus 58.3%, $p$ = 0.68; unadjusted HR 0.96 (95% CI 0.60–1.53), $p$ = 0.87) (Fig. 4). Adjusted HR for APACHE II was 1.06 (95% CI 0.66–1.69; $p$ = 0.81). Per-protocol analyses also revealed no difference between early and standard RRT group (HR for early RRT versus standard RRT 1.01 (95%CI 0.72-1.42), $p$ = 0.96). The mortality rate between RRT and no RRT in the standard RRT group was also not different (HR for RRT versus no RRT 1.59 (95% CI 0.85–4.97), $p$ =0.11). (Fig.5) Subanalysis did not reveal differences between mortality rates among each center ($p$ = 0.884) or between medical and surgical ICU ($p$=0.141).

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The 60-day mortality rate between early vs standard RRT group were not different (75.9% versus 71.7%, $p$ = 0.68). The 2-year mortality rate were also similar (84.5% versus 80%, $p$ = 0.63, unadjusted HR 1.13 (95% CI 0.75-1.68, $p$ = 0.56) (Fig.6).

*Figure 4* Survival curves of patients receiving early and standard renal replacement therapy (RRT) (straight line, early RRT group; dashed line, standard RRT group). The figure shows the Kaplan-Meier curve of the probability of survival from randomization to day 28. CI, confidence interval; HR, hazard ratio

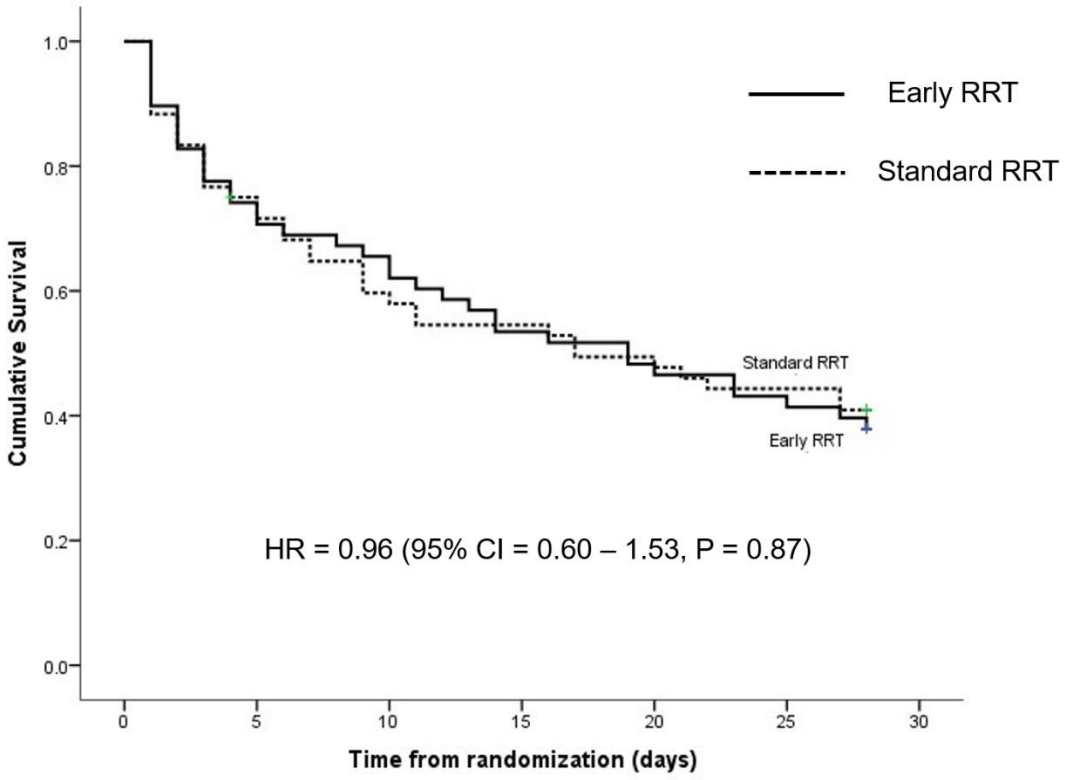


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*Figure 5* Survival curves of patients in the standard RRT arm who received and did not receive RRT (Blue line, No RRT group; red line, RRT group). The figure shows Kaplan-Meier curve of the probability of survival from randomization to day 28

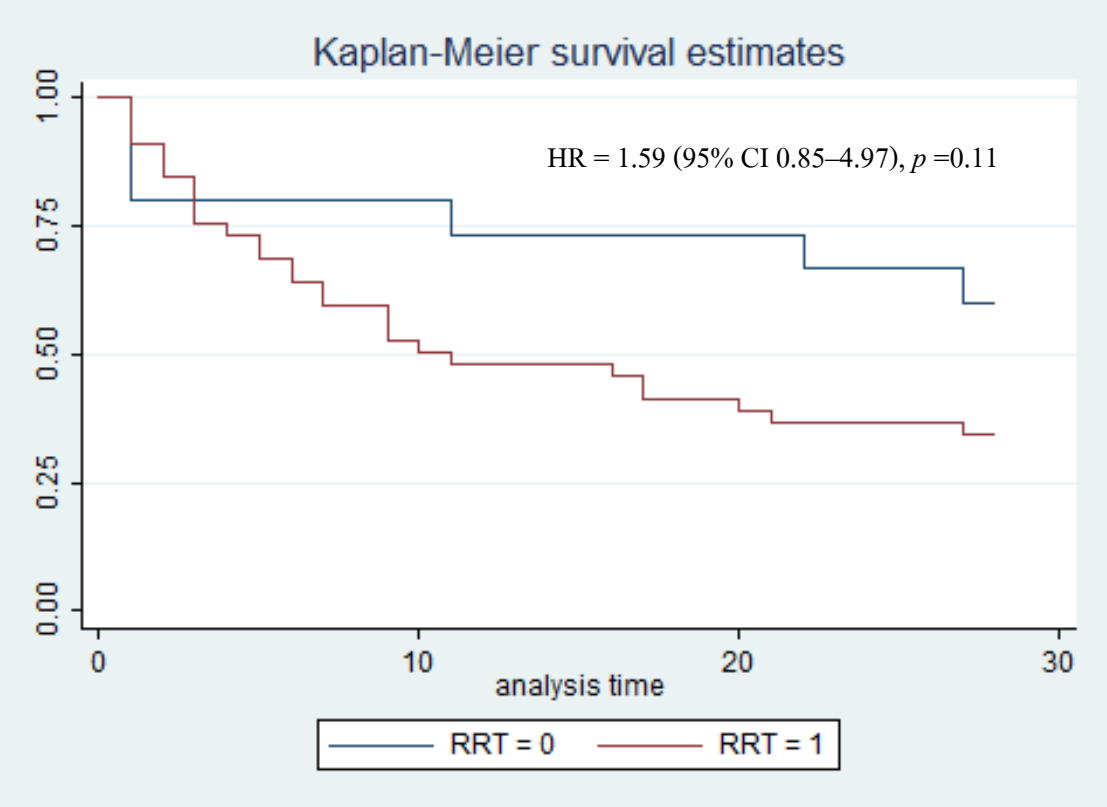


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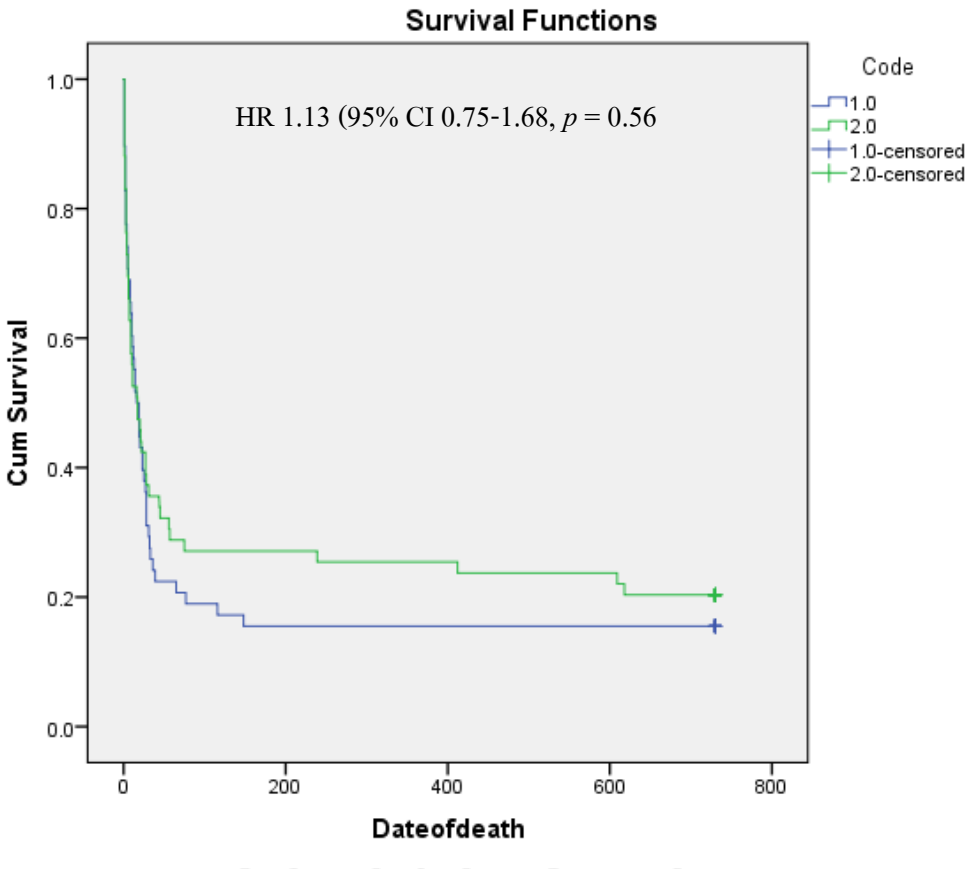
*Figure* 6 The 2-year survival curves of patients receiving early and standard renal replacement therapy (RRT) (straight line, early RRT group; dashed line, standard RRT group). The figure shows the Kaplan-Meier curve of the probability of survival from randomization to 2 years. CI, confidence interval; HR, hazard ratio



**Secondary outcomes**

There were no significant differences in renal recovery, cumulative fluid balance on the first 7 days, RRT-free days, mechanical ventilation-free days, ICU-free days, or dialysis dependence on day 28 between the two groups (Table 11). The levels of plasma NGAL, NT-proBNP, and angiopoietin-2 at the time of randomization were high. There were no significant differences in these three biomarkers on days 0, 3, and 7 within treatment arm and between treatment arms (Table 12).

*Table 11* Outcomes in Intervention trial

| Outcomes | Early RRT (n=58) | Standard RRT (n=60) | *p* value |
|---|---|---|---|
| Primary outcome | | | |
| Mortality, n (%) | 36 (62.1) | 35 (58.3) | 0.68 |
| Secondary outcomes | | | |
| Recovery, n (%) | 21 (36.2) | 19 (31.7) | 0.60 |
| 7-day fluid balance, mL, median [IQR] | -1702 [-5610-2129] | -1247 [-4535-1581] | 0.75 |
| Mean RRT dose, mL/kg/hr, mean (SD) | 26.8 (5.3) | 26.3 (8.9) | 0.73 |
| RRT-free days, days, median [IQR] | 0 [0-19] | 0 [0-28] | 0.64 |
| MV-free days, days, median [IQR] | 4 [0-24] | 0.5 [0-20.3] | 0.66 |
| ICU-free days, days, median [IQR] | 14 [0-21] | 4.5 [0-18] | 0.46 |
| ICU length of stay, days, median [IQR] | 12 [7-26] | 13.5 [9-29] | 0.76 |
| Hospital length of stay, days, median [IQR] | 26 [19-53] | 28.5 [17-55.3] | 0.82 |
| Renal replacement therapy dependency at day 28, n (%) | 7 (12.1) | 10 (16.7) | 0.77 |

RRT, renal replacement therapy; MV, mechanical ventilation; ICU, intensive care unit

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*Table 12* Comparison of severity score and plasma biomarkers from day 0, 3, and 7 in intervention trial

| Group | Early RRT | | | | Standard RRT | | | | *p* value+ |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Day 0 (N=58) | Day 3 (N=47) | Day 7 (N=40) | *p* value | Day 0 (N=60) | Day 3 (N=46) | Day 7 (N=39) | *p* value | |
| Non-renal SOFA score, mean (SD) | 9.9 (3.3) | 9.6 (4.1) | 8.1 (4.4) | 0.02 | 9.1 (4.1) | 7.8 (4.5) | 6.9 (4.4) | 0.03 | 0.09 |
| NGAL,++ ng/mL, median [IQR] | 894 [410.5-1456.8] | 969 [577-1827] | 1172.5 [424.7-2004] | 0.02 | 770 [439-1320] | 654 [364-1721] | 651 [256-1248] | 0.61 | 0.28 |
| NT-proBNP,+++ , pg/mL, median [IQR] | 4699 [920-35000] | 2545.9 [487.1-18484] | 2581 [581.3-21308] | 0.60 | 4231 [1684.3-13196] | 4884 [2024.3-20323] | 3070.2 [994.6-18325] | 0.46 | 0.48 |
| Ang2++++ , ng/mL, median [IQR] | 19077 [10528-41479] | 13561 [8053-33304] | 10653.5 [4936-14133] | <0.001 | 22829 [12096-34468] | 12920 [7822-31458] | 10476 [5677.5-22901] | 0.004 | 0.65 |
| Urine output, mL, median [IQR] | 407.5 [185-1123] | 129 [22-610] | 145 [10-1080] | 0.03 | 690 [247.5-1120] | 534 [65-2210] | 1385 [243-2100] | 0.25 | 0.039 |

+ Testing the difference of parameters between groups using generalized estimating equation (GEE)

++ NGAL, neutrophil gelatinase associated lipocalin

+++ NT-proBNP, N-terminal prohormone of brain natriuretic peptide

++++ Ang2, angiopoietin 2

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In the early RRT group, urine output in the early RRT group significantly decreased from day 0, 3, and 7. However, in the standard RRT group, urine output shows a trend for increase from day 0 to day 7. Compared between early and standard RRT group, change in urine output significantly differed between each group from day 0 to day 7.

**Adverse effects**

RRT-related and central venous catheter (CVC)-related adverse events are shown in Table 13. There was significantly more hypophosphatemia in the early RRT group ($p$ = 0.002). There were more CVC-related malfunctions and an incidence of air embolism in the early RRT group ($p$ = 0.038). Other RRT-related and CVC-related adverse events were comparable.

*Table 13* Adverse events in intervention trial

| Adverse events | Early RRT (n=58) | Standard RRT (n=60) | *p* value |
|---|---|---|---|
| Hemodynamic instability, n (%) | 20 (34.5) | 12 (20) | 0.08 |
| Arrhythmia, n (%) | 21 (36.2) | 16 (26.7) | 0.26 |
| Seizure, n (%) | 0 (0) | 0 (0) | 1.00 |
| Hypokalemia, n (%) | 3 (5.2) | 1 (1.7) | 0.29 |
| Hypophosphatemia, n (%) | 13 (22.4) | 2 (3.3) | **0.002** |
| Hypocalcemia, n (%) | 4 (6.9) | 4 (6.7) | 0.96 |
| CVC hemorrhage, n (%) | 1 (1.7) | 3 (5) | 0.33 |
| CVC pneumothorax, n (%) | 0 (0) | 0 (0) | 1.00 |
| CVC bacteremia, n (%) | 2 (3.4) | 2 (3.3) | 0.97 |
| CVC thrombosis, n (%) | 1 (1.7) | 0 (0) | 0.31 |
| Arterial puncture, n (%) | 2 (3.4) | 3 (5) | 0.68 |
| CVC others, n (%) | 4 (6.9)* | 0 (0) | 0.038 |

*Catheter malfunction 3 cases, air embolism 1 case

CVC, central venous catheter

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**Angiopoietin-2 and prediction of mortality in patients requiring RRT**

There were 86 patients with available blood samples on day 0, 3, and 7 (Fig. 7). We performed a substudy of the FST study to determine association between serum angiopoietin-2 and 28-day mortality. The mean age was 66.29 ± 15.78 years old; 52.3% were male and there was 60% mortality. Non-survivors had higher APACHE II and SOFA score, more proportions of hypertension and use of mechanical ventilators than survivors (Table 15). However, plasma NGAL and serum NT-proBNP on three days did not differ between survivors and non-survivors. Serum angiopoietin-2 was significantly higher in non-survivors than survivors on day 0, 3, and 7, p=0.02 (Fig. 8). By Spearman's correlation, serum angiopoietin-2 showed correlation with SOFA score (r=0.41, $p < 0.001$) (Table 16, Fig. 9).

Univariate and multivariate analysis showed significant associations between serum angiopoietin-2, APACHE II, and SOFA score and mortality (Table 17). Using a median cutoff of 20,540 ng/mL, patients with serum angiopoietin-2 more than this level had significantly lower survival than the lower group (Fig. 10). A 6,196 ng/mL level showed 100% sensitivity, 17.7% specificity, and 65.8% PPV, and 100% NPV in prediction of mortality. A 18,056 ng/mL level showed 67.3% sensitivity, 58.8% specificity, and 71.4% positive predictive value (PPV), and 54.1% negative predictive value (NPV) in prediction of mortality. Visualized by receiver operating curve, serum angiopoietin-2 showed area under the curve (AUC) of 0.63 (95% CI 0.506, 0.754), p = 0.045 compared with plasma NGAL, AUC-ROC of 0.608 (95% CI 0.484, 0.732), p = 0.906, for prediction of mortality (Fig. 11).

**Plasma NGAL and prediction of renal recovery in patients requiring RRT**

Renal recovery was defined by an increase of urine output to more than 1,000 mL without diuretics or more than 2,000 mL with diuretics and free from RRT for at least 7 days. We compared between patients with renal recovery and non-recovery at 28 days. Thirty out of 86 patients (34.9%) had renal recovery. There was a higher proportion of male, sepsis, and colistin use in non-recovery patients. Patients with non-recovery also had higher SOFA score than patients with renal recovery (Table 18). Median plasma NGAL at baseline and day 3 in non-

recovery patients were significantly higher than recovery patients (Table 18, Fig. 12). At baseline the median plasma NGAL level between recovery vs non-recovery were 496 ng/ml (interquartile range (IQR) 376–879) vs 1,047 ng/ml (IQR 587–1612), $p = 0.003$. On day 3, the median plasma NGAL level between recovery vs non-recovery were 577 ng/ml (interquartile range (IQR) 316–1,110) vs 1,398 ng/ml (IQR 643–2008), $p = 0.004$. Serum angiopoietin-2 and serum NT-proBNP did not differ between patients with recovery and non-recovery at all time points.

Univariate analysis showed that female sex, SOFA score, sepsis, RRT, log plasma NGAL at day 0 and 3, and plasma NGAL < 740 ng/mL were significantly associated with renal recovery, while multivariate analysis demonstrated significant association between plasma NGAL < 740 ng/mL, RRT, SOFA score, and female sex with renal recovery (Table 19). Using a median cutoff of 740 ng/mL, patients with plasma NGAL < 740 ng/mL had higher recovery by Kaplan-Meier curve (Fig. 13). A 740 ng/mL level showed 73.3% sensitivity, 69.8% specificity, 57.9% PPV, and 82.2% NPV in prediction of renal recovery. Visualized by receiver operating curve, plasma NGAL showed area under the curve (AUC) of 0.697 (95% CI 0.573, 0.821), $p = 0.003$ for prediction of renal recovery, compared with serum angiopoietin-2 at 0.594 (95% 0.469, 0.72), $p = 0.155$ (Fig. 14).

**Associated factors for RRT in the standard RRT group**

Among 60 patients in the standard RRT group, 45 (75%) patients underwent RRT by conventional indications compared with 15 (25%) patients with spontaneous renal recovery. Higher proportion of patients in the RRT group had sepsis (64.4% versus 20%, $p = 0.006$). Patients in the RRT group also had higher APACHE II, SOFA score, baseline plasma NGAL and NT-proBNP compared with non-RRT group (Table 20). Univariate analysis showed significant associations between SOFA score, sepsis, and baseline NT-proBNP with RRT. After multivariate analysis, plasma NGAL had HR 1.06 (95% CI 1.01-1.12; $p = 0.024$) adjusted with SOFA score and sepsis (Table 21). Plasma NGAL alone showed AUC-ROC of 0.707 (95% CI 0.522,0.891) for prediction of RRT, but increased to 0.781 (95% CI 0.594, 0.967) when combined with baseline NT-proBNP, and increased to 0.794 (95% CI 0.613, 0.974) when combined with clinical parameters including SOFA score and sepsis (Fig.15).

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# CHAPTER V: DISCUSSION

In this randomized controlled trial (RCT), we demonstrated the feasibility and safety of conducting a trial comparing early versus standard RRT using FST as an initial triage strategy. The results of the present study demonstrate that FST was easy to administer in the context of a clinical trial (100% compliance) and provided excellent predictive ability for the subsequent use of RRT; nonresponsive patients had an RRT rate of 75% versus 13.6% for FST-responsive patients (Table 7). Compliance with other aspects of the study protocol was also excellent, with > 95% of patients receiving the intervention they were randomized to receive. Randomization was successful in that baseline characteristics were well-balanced between intervention arms except APACHE II score which was higher in the early RRT group. Finally, we achieved excellent follow-up, with 100% of patients available for survival analysis.

We did not encounter any safety issues using the FST, and the only adverse events encountered with early initiation were increased rates of hypophosphatemia and dialysis catheter issues (Table 13). We chose 6 hours after FST administration as a cutoff for early RRT to provide timeframe for catheter insertion and circuit initiation and to achieve adequate separation from the standard RRT group. Timing of initiation in the early group and the standard group were 2 and 21 hours, which approached the difference of 24 hours between the two groups. When considering from ICU admission to RRT, timing between early and standard RRT was 22 and 100 hours, respectively, which may be considered relatively late. However, when considering timing from oliguria to RRT, the timing between early and standard RRT was 17 and 38 hours, with the 21-hour difference similar to timing from FST. It should be noted that patients may have different onsets of AKI and may result in different stagings when FST was done.

Our samples may have been too small to detect the difference in mortality rates and secondary outcomes. When we first calculated the sample size, we approximated the 39 percent difference in mortality rates by using the results from the ELAIN study.[34] The final results are only 4% difference in mortality rates, calculated back to 6.7% power (Table 11, Fig. 4). When using this preliminary data with 80% power, the new sample size would be 5,600 patients. Therefore, our final sample size was insufficient to test whether timing of initiation of RRT impacted 28-day survival. Other secondary outcomes including renal recovery rate, ICU-free

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days, mechanical ventilator-free days, 7-day fluid balance, and dialysis dependence rate were not significantly different between both treatment arms (Table 11), although we were unpowered for many of these endpoints. For example, ventilator-free days and ICU-free days were both greater with early initiation but with very wide confidence intervals. Similarly, the small differences observed in ICU and hospital length of stay (difference of 1.5 and 2.5 days, respectively) are clinical relevant but would have required a much larger trial to detect. Plasma NGAL in the early RRT group on day 3 and day 7 in the early RRT seemed to be higher than the standard RRT group, probably explained by exposure to extracorporeal circuits and membraine-induced inflammation. Interestingly, urine output in the early RRT group significantly decreased from day 0 to 7, but increased in the standard RRT group. The decreased amount of urine output explained by exposure to extracorporeal circuit could affect time to renal recovery although in our study the percentage of renal recovery and dialysis dependence at 28-day did not differ between each group. There was more hemodynamic instability (34.5% versus 20%) with early initiation while less cumulative fluid removal was seen at 7 days with standard RRT (1.2 versus 1.7 L), but neither of these differences were significant. However, there was a significant difference in fluid accumulation from randomization to initiation of RRT (4.8 versus 8.7 L, $p = 0.02$). Finally, there was more hypophosphatemia in the early RRT group, explained by removal of small molecules by CRRT circuit[46].

The optimal timing to initiate RRT in AKI patients remains to be established. [13, 32-35, 47-54] Three recently published RCTs examining timing of RRT initiation reached different conclusions. The AKIKI multicenter trial in France investigated early initiation (within 6 h after documentation of KDIGO stage 3) versus a "wait and see" strategy (as per conventional indications). Sepsis, severe sepsis, or septic shock were present in 80%. Mortality at 60 days was not different between the two strategies. [35] The IDEAL-ICU similarly included only sepsis patients and used RIFLE "Failure" criteria for early initiation and conventional indications or anuria for 48 hours for standard initiation. The 90-day mortality rate also did not differ between two groups. [33] Conversely, a single center RCT in Germany (ELAIN study) defined early RRT as AKI KDIGO stage 2 plus plasma NGAL > 150 ng/mL and delayed RRT as AKI stage 3. Early initiation of RRT significantly reduced 90-day mortality compared with delayed initiation (39.3% versus 54.7%).[34] Comparison between each study and the FST study is shown in Table 14. The

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other ongoing study, Standard versus Accelerated initiation of Renal Replacement Therapy in Acute Kidney Injury (STARRT-AKI), also uses the higher cut-off level of plasma NGAL (>= 400 ng/mL) as one of the three inclusion criteria along with a twofold rise in serum creatinine and oliguria.[55] By using plasma NGAL as a screening biomarker to filter patients, the ELAIN trial was able to select 90% of patients in the standard arm who required RRT. On the contrary, 49% of the patients in the standard indication arm of the AKIKI trial and 29% of those of the IDEAL-ICU trial, which used only AKI staging as a screening tool, showed spontaneous recovery, which implied that RRT could also be avoided in some patients in the early indication arm had there been screening tools for selection of high-risk patients. Therefore, a pure clinical strategy may not be enough to analyze early versus standard initiation strategy and prevent unnecessary RRT.

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*Table 14* Comparison between AKIKI, ELAIN, and IDEAL-ICU trial and our study (FST study)

| Studies | AKIKI[35] | ELAIN[34] | IDEAL-ICU[33] | **FST study** |
|---|---|---|---|---|
| No. | 620 | 231 | 488 | 118 |
| Population | Sepsis 79.5% | Surgery 94.8% | Sepsis 100% | Sepsis 60% |
| Enrollment criteria | KDIGO staging | KDIGO staging | RIFLE criteria | Nonresponsive to FST |
| Early | KDIGO stage 3 | KDIGO stage 2 | Failure stage | Within 6 hours |
| Standard | By indications | KDIGO stage 3 | Emergency conditions 48 hours after AKI Diagnosis | By indications |
| Received RRT in standard group | 51% | 90.8% | 62% | 75% |
| Mode of RRT | IHD 50%, CRRT 30% | CRRT 100% | IHD 34%, CRRT, 46%, Both 20% | CRRT 100% |
| Primary endpoint | 60-d mortality 49% vs 50% (NS) | 90-d mortality 39% vs 55% (p = 0.03) | 90-d mortality 58% vs 54% (NS) | 28-d mortality 62% vs 58% (NS) |
| Secondary endpoints | *Early strategy* Delayed diuresis Higher catheter-related bloodstream infections | *Early strategy* Shorter RRT duration and hospital length of stay | *Early strategy* Shorter RRT-free days | *Early strategy* Higher hypophosphatemia Comparable changes in biomarkers |

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In our study, we identified 44/162 (27.2%) FST-responsive patients with only a 13.6% rate of RRT. RRT was averted in 86.4% of FST responders. We were able to select a group with a 75% RRT rate in the standard RRT group of FST nonresponders. In the standard arm, plasma NGAL was also a significant predictor for RRT requirement. This is similar to another study by our group (EARLY-RRT) which employed plasma NGAL for stratification of high-risk patients who would likely need RRT. Patients who reached plasma NGAL of at least 400 ng/mL was randomized to early and standard RRT group. We found out that plasma NGAL had a very high negative predictive value, with 0% of patients who had plasma NGAL lower than 400 ng/mL eventually requiring RRT. However, the positive predict value is only fair, with 40% of patients with plasma NGAL higher than 400 ng/mL requiring RRT.[37]

This suggests that FST is an excellent strategy to select patients who would likely require RRT and plasma NGAL had high-yield for excluding patients who were unlikely to require RRT. Combining FST nonresponsiveness with plasma NGAL might be an even more suitable strategy to predict patients who are likely to require RRT.

Our study introduces a novel concept in RRT initiation. There have been many large RCTs which used different criteria for early and standard RRT, included different subgroups of population, and employed heterogeneous modes of RRT. In the AKIKI and IDEAL-ICU studies, there were a significant number of patients in the standard RRT group who did not require RRT. This implies that even in severe AKI, early initiation could expose patients to unnecessary RRT. Moreover, the mortality rate in the standard RRT group-no RRT versus standard RRT group-RRT were 37.1% versus 61.8% in the AKIKI trial. [35] In our study, the mortality rate in the no RRT versus RRT group was 40% versus 64.4%, which were similar to the AKIKI study. While there is no strong evidence to support early initiation of RRT, initiation of RRT by emergency conditions could risk the patients to AKI complications resulting in highest mortality. There needs to be a tool to stratify patients if they would require RRT or not, then a judicious " watch and wait" strategy could be used for timely RRT initiation. Our study uses furosemide, a widely available drug with uncomplicated administration protocol, which shows excellent ability to select patients who would require RRT. This is particularly useful in Thailand, where biomarkers such as NGAL are not generally available. General practitioners, internists, intensivists, and nephrologists can adopt this protocol in their daily routines and sort out only

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high-risk patients. This could tremendously save the resources for patients' care, referral, and RRT. Notably, furosemide responsiveness is influenced by serum albumin and albuminuria. Therefore, application of FST in real-life practice needs consideration of these two factors cautiously. Further studies should compare between routine use of FST in practice and standard RRT initiation practice for mortality, RRT rates, complications, and cost-effectiveness.

For the substudy including only patients with available blood samples at 3 time points, we explored the association between each biomarker and mortality. Serum angiopoietin-2 was significantly higher in non-survivors than survivors and showed significant associations with 28-day mortality. Serum angiopoietin-2 inhibits Tie2/Angiopoietin-1 axis which regulates vascular endothelial integrity, blood vessel remodeling, and maturation. Inflammation stimulates angiopoietin-2, thereby promoting endothelial leakage, vascular permeability, and vascular inflammation.[45] Previous studies have shown associations of serum angiopoietin-2 and acute lung injury, hepatic dysfunction, coagulopathy, acute kidney injury, and multiorgan dysfunction in critically ill patients.[56-60] Our results supported association of angiopoietin-2 and increased mortality in critically ill patients during renal replacement therapy and showed a fair predictive value for mortality. Interestingly, angiopoietin-2 correlated with SOFA score but not fluid status nor sepsis. This can be implied that endothelial dysfunction might reflect the patients' severity and multiorgan failure more than fluid overload or sepsis, which might also contributes to increased vascular permeability.

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Our study also showed modest predictive value for renal recovery with baseline plasma NGAL. This is consistent with another study by Srisawat et al. demonstrating plasma NGAL as a predictive factor for renal recovery in pneumonia-associated acute kidney injury. In this study, a cut-off point of 393 ng/mL showed a 47% sensitivity, 90% specificity with a positive and negative predictive value of 83% and 63%, respectively.[61] Plasma NGAL and negative association with recovery could be related to increased production, decreased elimination, or both. Higher pNGAL concentration following AKI may be because of increased NGAL production in the distant organs e.g. liver and lung and its release into the circulation following AKI, increased NGAL production from the neutrophils and inflammatory cells, or the decreased elimination of pNGAL because of a decrease in glomerular filtration rate after AKI.[62, 63] Our study uses higher cutoff value for prediction of renal recovery, probably reflecting patients with

higher severity and inflammatory states, i.e. higher SOFA score and renal replacement therapy rate than the previous study.

There are some limitations in our study. First, due to the nature of the study, this was an unblinded RCT. The robust protocol for initiation of RRT and high compliance rates minimizes the risk of bias in RRT initiation. Second, the numbers of participants were rather small (60 in each arm) leading to insufficient power for secondary endpoints. However, as a pilot study, our results support the feasibility and safety of this approach for a definitive trial in the future. The incidence of hypophosphatemia was higher in the early RRT group, and severe hypophosphatemia is known to be associated with respiratory failure and weaning failure [64]. Plasma NGAL, NT-proBNP, and angiopoietin-2 levels in the early intervention arm were not significantly different from standard RRT. However, there were wide confidence intervals and important differences could have been missed.

In conclusion, the FST study shows that furosemide stress test had an excellent ability to stratify patients who would require RRT. Early initiation of RRT had not been shown to demonstrate different 28-day mortality rates than standard initiation of RRT. Our study supports the feasibility of using FST as a novel biomarker to guide implementation of large-scale RCTs for the timing of RRT initiation.

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## SUPPLEMENTARY MATERIAL

*Figure 7* Flow chart for patients with available blood samples

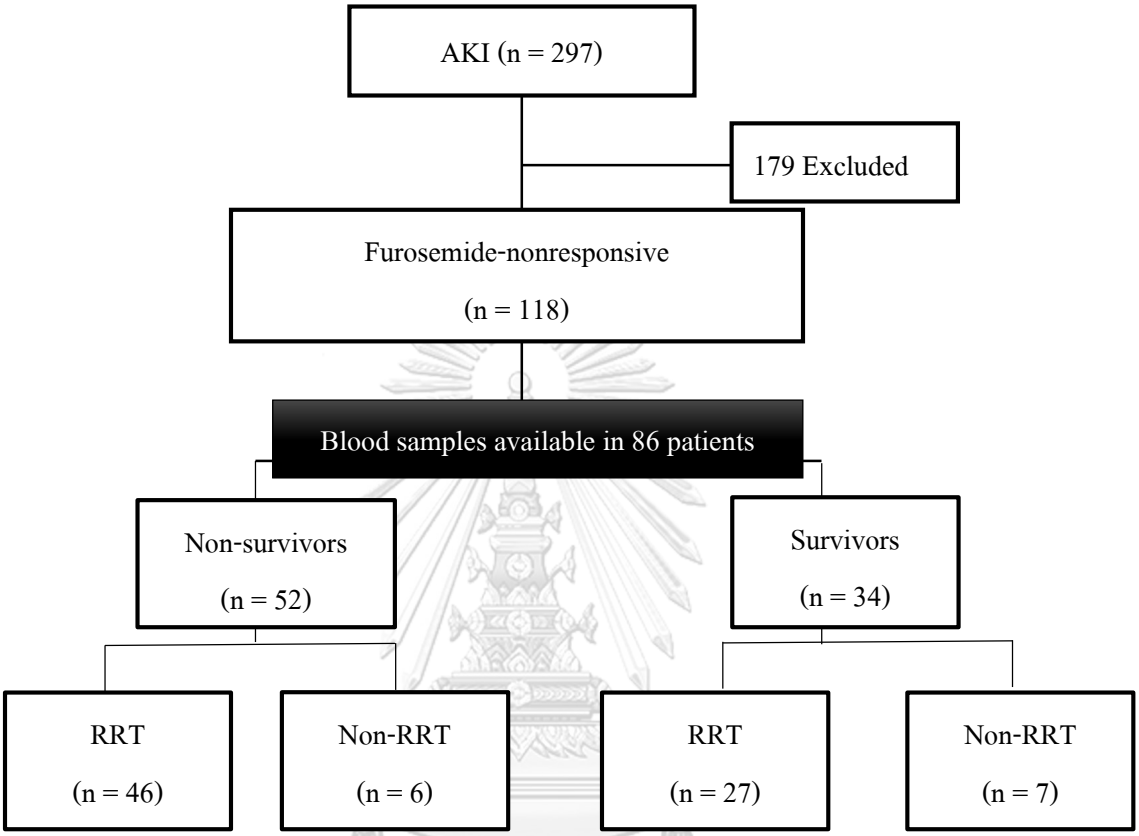


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*Table 15* Baseline characteristics and biochemical parameters during day 0, 3, and 7 between survivors and non-survivors

| Parameters | Non-survivors (n=52) | Survivors (n=34) | *p value* |
|---|---|---|---|
| Medical ICU | 35 (67.3%) | 21 (61.8%) | 0.598 |
| Surgical ICU | 17 (32.7%) | 13 (38.2%) | |
| Age | 64.79 ± 17.22 | 68.59 ± 13.2 | 0.396 |
| Male | 29 (55.8%) | 16 (47.1%) | 0.429 |
| Weight | 67.31 ± 30.52 | 59.07 ± 13.76 | 0.203 |
| APACHE II | 24.9 ± 6.89 | 21.15 ± 6 | **0.008** |
| SOFA | 12.85 ± 3.63 | 11 ± 3.07 | **0.009** |
| Non-renal SOFA | 10.46 ± 3.77 | 8.38 ± 3.03 | **0.007** |
| Baseline creatinine (mg/dL) | 1.03 ± 0.33 | 1.01 ± 0.37 | 0.856 |
| eGFR (mL/min/1.73m$^2$) | 72.37 ± 25.84 | 69.94 ± 24.7 | 0.727 |
| AKI staging | | | |
| 1 | 13 (25%) | 5 (14.7%) | 0.195 |
| 2 | 20 (38.5%) | 10 (29.4%) | |
| 3 | 19 (36.5%) | 19 (55.9%) | |
| Hypertension | 22 (42.3%) | 22 (64.7%) | **0.042** |
| Diabetes | 13 (25%) | 8 (23.5%) | 0.877 |
| Dyslipidemia | 13 (25%) | 13 (38.2%) | 0.191 |
| Ischemic heart disease | 9 (17.3%) | 9 (26.5%) | 0.307 |
| Malignancy | 12 (23.1%) | 4 (11.8%) | 0.187 |
| Cerebrovascular disease | 3 (5.8%) | 5 (14.7%) | 0.163 |
| Chronic liver disease | 7 (13.5%) | 8 (23.5%) | 0.229 |
| Mechanical ventilation | 47 (90.4%) | 25 (73.5%) | **0.038** |
| Vasopressors | 45 (86.5%) | 25 (73.5%) | 0.130 |
| Sepsis | 32 (61.5%) | 17 (50%) | 0.291 |
| Nephrotoxic agents | 15 (28.8%) | 13 (38.2%) | 0.364 |
| Cardiac surgery | 8 (15.4%) | 7 (20.6%) | 0.534 |
| Percent of fluid overload | 7.71 (3.91, 15.1) | 5.44 (2.76, 13.37) | 0.244 |
| NGAL day 0 (ng/mL) | 1010 (512, 1645) | 635.5 (401, 1310) | 0.096 |

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| Parameters | Non-survivors (n=52) | Survivors (n=34) | p-value |
|---|---|---|---|
| NGAL day 3 (ng/mL) | 1122 (376, 2064) | 738 (386, 1586) | 0.294 |
| NGAL day 7 (ng/mL) | 691.2 (301.8, 1380) | 847 (404, 1997) | 0.364 |
| NT-proBNP day 0 (pg/mL) | 5009.5 (1540.7, 26647.5) | 4612 (1243, 9978) | 0.446 |
| NT-proBNP day3 (pg/mL) | 5455 (601.5, 22763) | 4395 (949.4, 16140) | 0.723 |
| NT-proBNP day7 (pg/mL) | 1817 (581.3, 21308) | 3441 (639.6, 18493) | 0.832 |
| Ang-2 day 0 (ng/mL) | 24995 (15802.5, 42908.5) | 16277 (9128, 32759) | **0.023** |
| Ang-2 day 3 (ng/mL) | 19989 (10245, 37595) | 10516 (7644, 22803) | **0.013** |
| Ang-2 day 7 (ng/mL) | 14769 (8815, 20641) | 7715 (5126, 13115) | **0.033** |
| Renal replacement therapy | 46 (88.5%) | 27 (79.4%) | 0.252 |
| Time from randomization to RRT (hours) | 4.5 (1, 16) | 10 (2, 21) | 0.350 |
| Time from ICU admission to RRT (hours) | 32 (16, 129) | 44 (14, 121) | 0.991 |
| Time from oliguria to RRT (hours) | 20.5 (15, 31) | 30 (16, 45) | 0.191 |
| Fluid accumulation at RRT (mL) | 7185.5 (3266, 9383) | 3557 (1381, 9567) | 0.139 |

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*Figure 8* Median levels of serum angiopoietin-2 during day 0, 3, and 7 between survivors and non-survivors

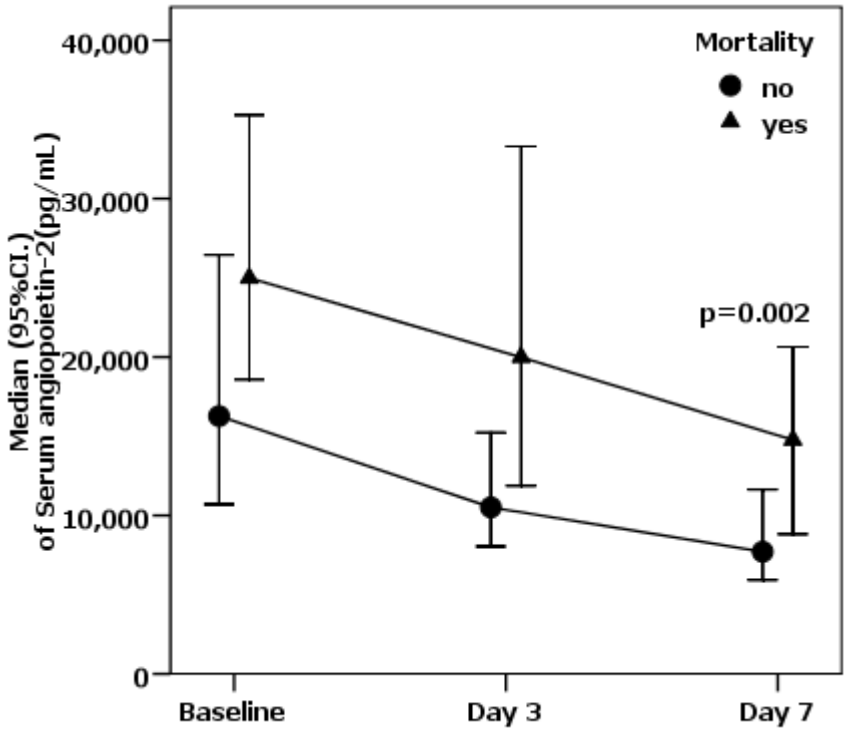


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*Table 16* Correlation between serum angiopoietin-2 at day 0 and clinical parameters

| Parameters | Serum angiopoietin-2 (ng/mL) | |
|---|---|---|
| | r | p-value |
| APACHEII | 0.182 | 0.093 |
| SOFA | 0.410 | **<0.001** |
| AKI staging | 0.135 | 0.214 |
| Fluid balance at randomization | 0.018 | 0.869 |
| Percent of fluid overload | -0.047 | 0.667 |
| Sepsis | 0.305 | 0.106 |

Spearman's rho correlations

*Figure 9* Spearman's correlation shows significant correlation between serum angiopoietin-2 and SOFA score

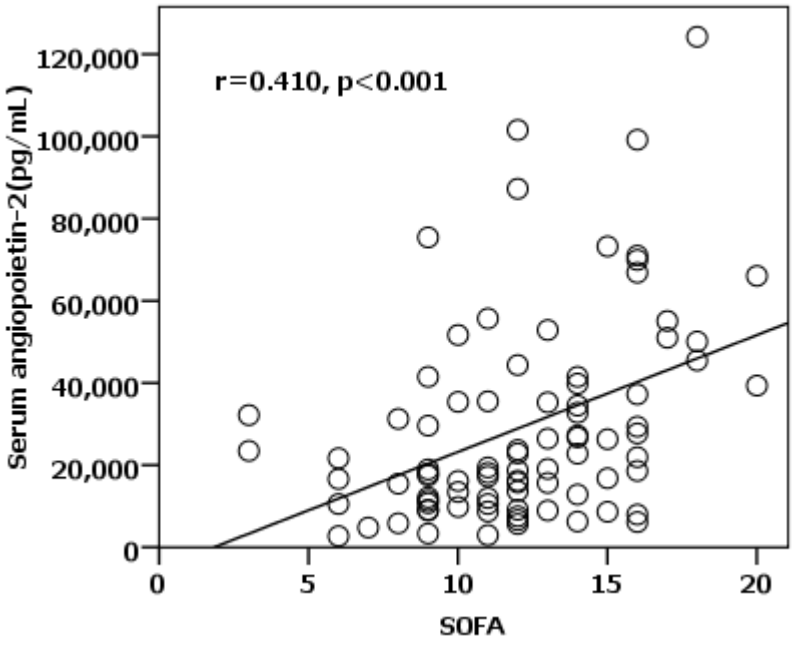

*Table 17* Univariate and multivariate analysis for associated factors with mortality

| Variables | Unadjusted HR | 95% CI | *p value* | Adjusted HR | 95% CI | *p value* |
|---|---|---|---|---|---|---|
| Male | 1.33 | 0.77-2.28 | 0.304 | | | |
| Age | 0.91 | 0.77-1.08 | 0.278 | | | |
| Surgical ICU | 1.13 | 0.63-2.01 | 0.682 | | | |
| Received RRT | 1.38 | 0.62-3.06 | 0.43 | | | |
| Cardiac surgery | 0.83 | 0.39-1.76 | 0.626 | | | |
| Use of nephrotoxic agents | 0.84 | 0.46-1.52 | 0.555 | | | |
| Sepsis | 1.35 | 0.77-2.36 | 0.284 | | | |
| Mechanical ventilator | 2.46 | 0.96-6.17 | 0.056 | | | |
| Vasopressors | 1.88 | 0.85-4.16 | 0.122 | | | |
| Fluid overload | 1.12 | 0.82-1.52 | 0.485 | | | |
| Plasma NGAL per 1000 ng/mL increase | 1.27 | 0.98-1.63 | 0.071 | | | |
| Serum NT-proBNP per 1,000 pg/mL increase | 1.01 | 0.99-1.03 | 0.501 | | | |
| Serum angiopoietin-2 per 5000 ng/mL increase | 1.07 | 1.02-1.12 | **0.008** | 2.61 | 1.12-6.06 | **0.026** |
| APACHE II | 1.06 | 1.01-1.10 | **0.01** | 1.04 | 0.99-1.09 | 0.15 |
| SOFA | 1.11 | 1.03-1.21 | **0.01** | 1.02 | 0.92-1.13 | 0.68 |
| AKI staging | | | | | | |
| Stage 1 | 1.52 | 0.76-3.06 | 0.329 | | | |
| Stage 2 | 1.38 | 0.73-2.60 | 0.235 | | | |
| Stage 3 | Reference | | | | | |

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*Figure 10* Kaplan Meier survival curve shows lower survival rates divided by median level of serum angipoietin-2 levels (20,540 ng/mL)

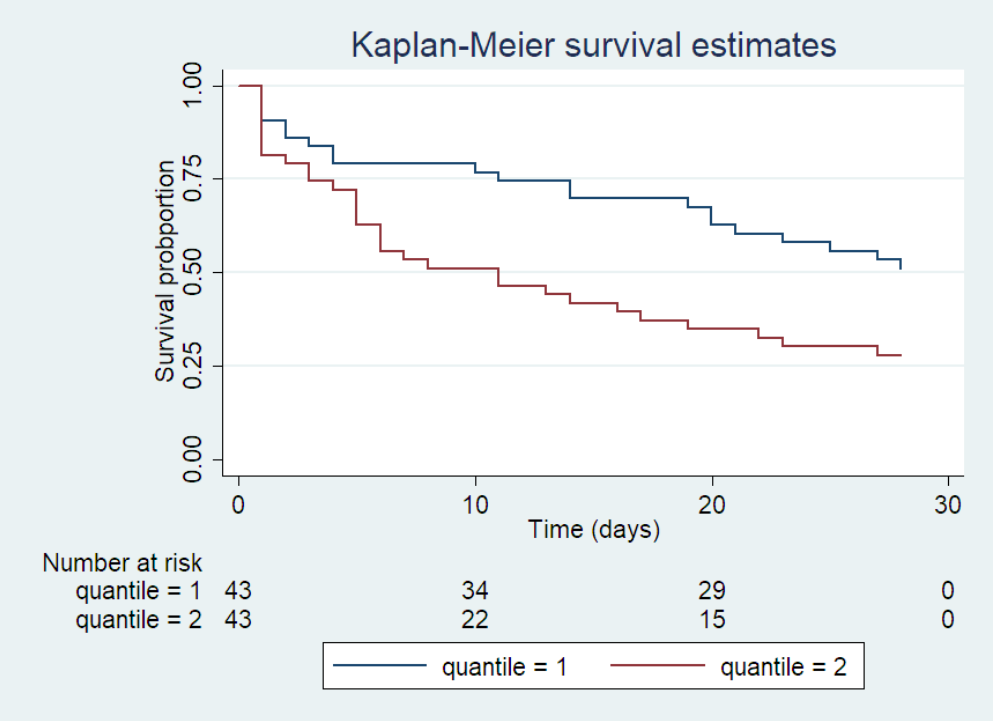


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*Figure 11* AUC-ROC of baseline serum angiopoietin-2 and plasma NGAL for prediction of mortality

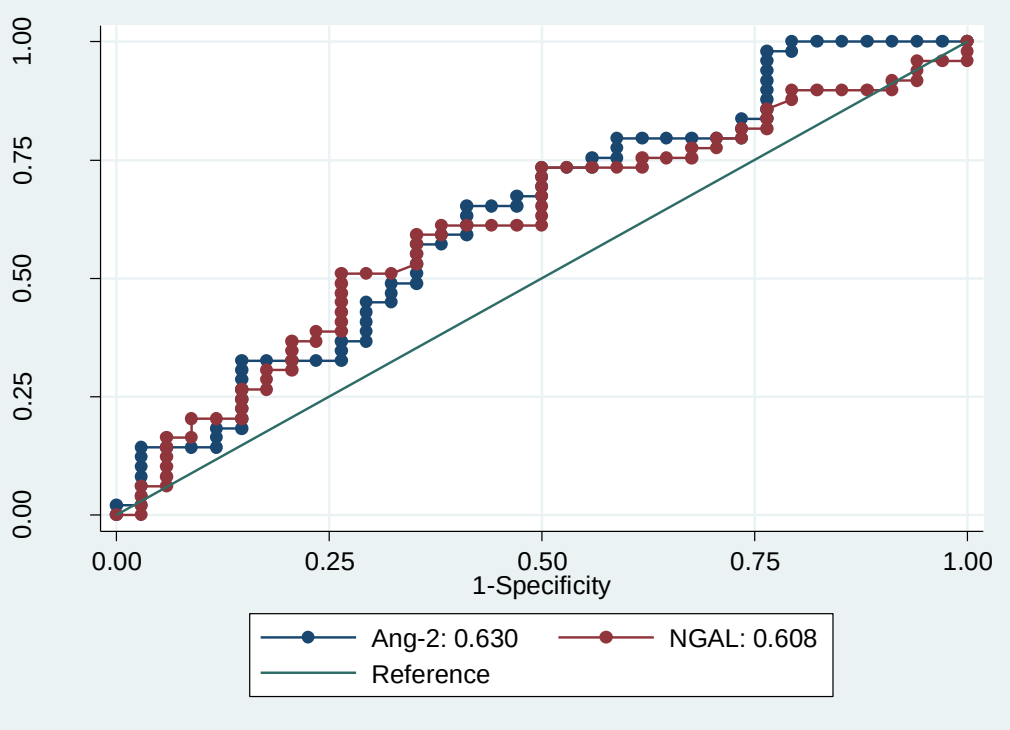


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*Table 18* Baseline characteristics and biochemical parameters during day 0, 3, and 7 between recovery and non-recovery

| Parameters | Recovery (n=30) | Non-recovery (n=56) | p-value |
|---|---|---|---|
| ICU | | | |
| Medical ICU | 18 (60%) | 38 (67.9%) | 0.466 |
| Surgical ICU | 12 (40%) | 18 (32.1%) | |
| Age | 70.33 ± 15.55 | 64.13 ± 15.61 | 0.115 |
| Male | 11 (36.7%) | 34 (60.7%) | **0.033** |
| Weight | 58.78 ± 13.61 | 66.88 ± 29.7 | 0.142 |
| APACHE II | 21.97 ± 6.39 | 24.2 ± 6.9 | 0.217 |
| SOFA | 10.87 ± 3.5 | 12.79 ± 3.38 | **0.011** |
| Nonrenal SOFA | 8.27 ± 3.48 | 10.37 ± 3.51 | **0.013** |
| Cr baseline (mg/dL) | 1.02 ± 0.37 | 1.02 ± 0.34 | 0.996 |
| eGFR (mL/min/1.73m$^2$) | 66.3 ± 22.25 | 74.14 ± 26.54 | 0.252 |
| AKI staging | | | |
| 1 | 5 (16.7%) | 13 (23.2%) | 0.466 |
| 2 | 13 (43.3%) | 17 (30.4%) | |
| 3 | 12 (40%) | 26 (46.4%) | |
| Hypertension | 16 (53.3%) | 28 (50%) | 0.768 |
| Diabetes | 8 (26.7%) | 13 (23.2%) | 0.722 |
| Dyslipidemoa | 8 (26.7%) | 18 (32.1%) | 0.598 |
| Ischemic heart disease | 9 (30%) | 9 (16.1%) | 0.130 |
| Malignancy | 4 (13.3%) | 12 (21.4%) | 0.358 |
| Cerebrovascular diseases | 2 (6.7%) | 6 (10.7%) | 0.538 |
| Chronic liver disease | 7 (23.3%) | 8 (14.3%) | 0.292 |
| Mechanical ventilators | 23 (76.7%) | 49 (87.5%) | 0.195 |
| Vasopressors | 22 (73.3%) | 48 (85.7%) | 0.160 |
| Sepsis | 12 (40%) | 37 (66.1%) | **0.020** |
| Nephrotoxic | 7 (23.3%) | 21 (37.5%) | 0.181 |
| Colistin | 1 (3.3%) | 12 (21.4%) | **0.026** |
| Vancomycin | 0 (0%) | 2 (3.6%) | 0.295 |

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| Parameters | Recovery (n=30) | Non-recovery (n=56) | p-value |
|---|---|---|---|
| Contrast | 7 (23.3%) | 9 (16.1%) | 0.409 |
| Aminoglycoside | 1 (3.3%) | 2 (3.6%) | 0.954 |
| Amphotericin B | 0 (0%) | 1 (1.8%) | 0.462 |
| Cardiac surgery | 7 (23.3%) | 8 (14.3%) | 0.292 |
| Percent of fluid overload | 7.65 (3.29, 15.64) | 6.91 (3.67, 12.92) | 0.821 |
| NGAL day 0 (ng/mL) | 495.5 (376, 879) | 1047 (587, 1612) | **0.003** |
| NGAL day 3 (ng/mL) | 577 (316, 1110) | 1397.5 (642.7, 2008) | **0.004** |
| NGAL day 7 (ng/mL) | 596 (301.6, 1504) | 1090 (474.45, 2004.5) | 0.075 |
| NT-proBNP day 0 (pg/mL) | 3855.9 (867.3, 27544) | 5019 (1792, 22106) | 0.493 |
| NT-proBNP day3 (pg/mL) | 3055 (949.4, 16048) | 5455 (601.5, 29111) | 0.500 |
| NT-proBNP day7 (pg/mL) | 1817 (639.6, 21308) | 4879 (787.2, 18325) | 0.869 |
| Ang-2 day 0 (ng/mL) | 18030.5 (9797, 29590) | 23250.5 (14566, | 0.087 |
| Ang-2 day 3 (ng/mL) | 11922 (7644, 22803) | 19232 (9825, 36516) | 0.084 |
| Ang-2 day 7 (ng/mL) | 9311 (4790, 13379) | 11641 (7165, 20515) | 0.138 |
| RRT | 21 (70%) | 52 (92.9%) | **0.005** |

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*Figure* 12 Median levels of plasma NGAL during day 0, 3, and 7 between renal recovery and non-recovery

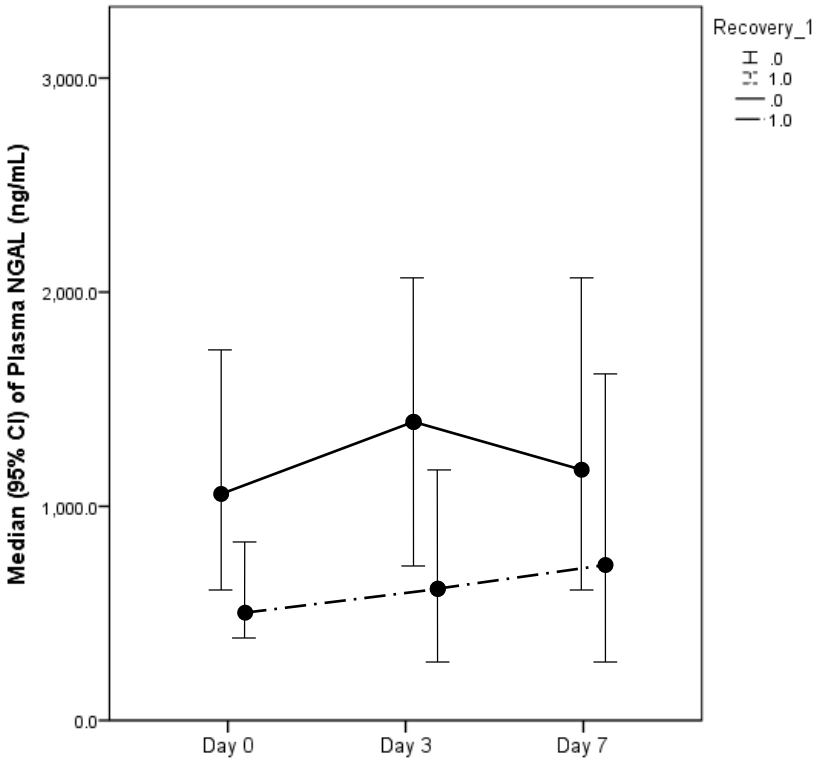


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*Table* 19 Univariate and multivariate analysis for associated factors with renal recovery

| Parameters | Unadjusted HR | p-value | Adjusted HR (95%CI) | p-value |
|---|---|---|---|---|
| Age | 1.03 (1, 1.06) | 0.085 | | |
| Female sex | 2.67 (1.07, 6.67) | **0.036** | 2.04 (1.01, 2.86) | **0.05** |
| SOFA | 0.85 (0.74, 0.97) | **0.019** | 0.91 (0.83, 1.00) | **0.05** |
| Ischemic heart disease | 2.24 (0.78, 6.44) | 0.135 | | |
| Vasopressors | 0.46 (0.15, 1.38) | 0.165 | | |
| Sepsis | 0.34 (0.14, 0.86) | **0.022** | 0.76 (0.37,1.54) | 0.451 |
| Nephrotoxic | 0.51 (0.19, 1.38) | 0.185 | | |
| Colistin | 0.13 (0.02, 1.03) | 0.053 | | |
| RRT | 0.18 (0.05, 0.65) | **0.009** | 0.39 (0.17, 0.90) | **0.027** |
| NGAL baseline <740 | 6.36 (2.34, 17.28) | **<0.001** | 2.70 (1.41, 5.10) | **0.003** |
| LogNGAL_at baseline | 0.47 (0.24, 0.91) | **0.025** | | |
| LogNGAL day 3 | 0.33 (0.17, 0.66) | **0.002** | | |
| LogNGAL day 7 | 0.58 (0.30, 1.13) | 0.11 | | |

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*Figure* 13 Kaplan Meier survival curve comparing time to renal recovery divided by median level of plasma NGAL levels (740 ng/mL)

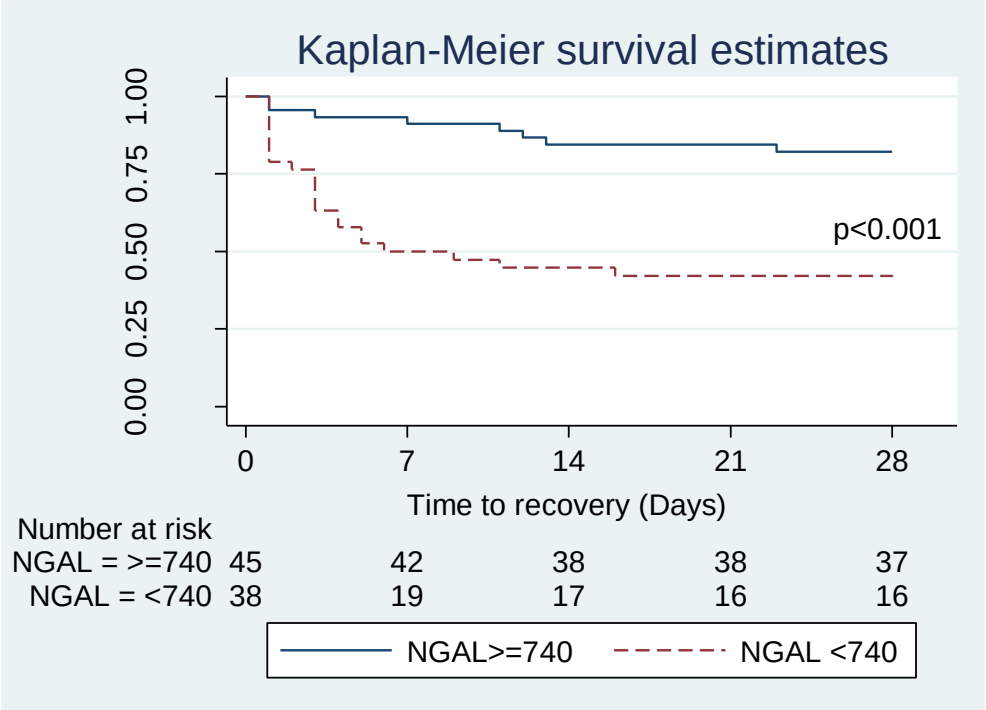


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*Figure* 14 AUC-ROC of baseline serum angiopoietin-2 and plasma NGAL for prediction of renal non-recovery

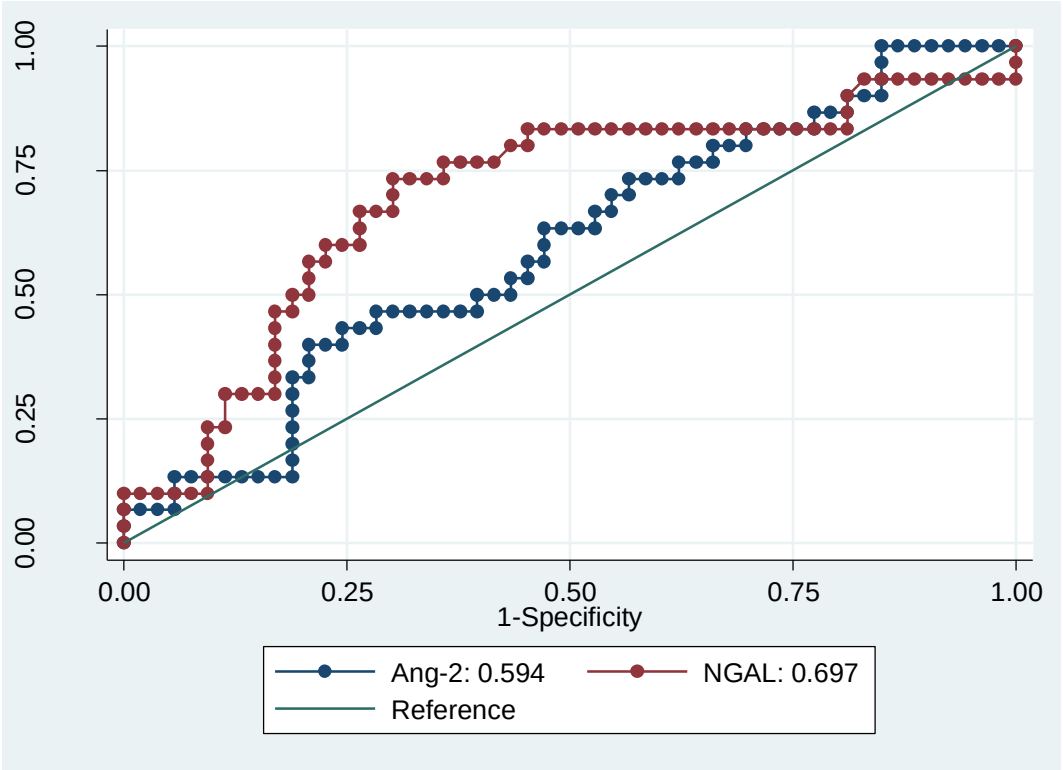


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*Table 20* Baseline characteristics and biochemical parameters during day 0, 3, and 7 between RRT and no RRT groups

| | RRT (n=45) | No RRT (n=15) | *p* value |
|---|---|---|---|
| Age | 66.7 ± 16.2 | 66.6 ± 18.5 | 0.98 |
| Male (%) | 55.6 | 26.7 | 0.075 |
| APACHE II | 22.8 ± 6.5 | 18.7 ± 7.2 | **0.04** |
| SOFA score | 12.1 ± 3.7 | 9.3 ± 4.04 | **0.02** |
| CKD (%) | 37.8 | 40 | 1.00 |
| AKI stage 1 | 53.3 | 53.3 | 1.00 |
| 2 | 26.7 | 26.7 | |
| 3 | 20 | 20 | |
| Sepsis (%) | 64.4 | 20 | **0.006** |
| MV (%) | 88.9 | 66.7 | 0.102 |
| Vasopressors (%) | 80 | 73.3 | 0.72 |
| Fluid accumulation (mL) | 4002 (2319-8826) | 5280 (2277-6758) | 0.87 |
| Plasma NGAL day 0 (ng/mL) | 885.5 (450–1320) | 518.5 (397.5–641.5) | **0.09** |
| Serum NT-proBNP day 0 (pg/mL) | 6718 (2263-22106) | 1582 (864-4526) | **0.02** |
| Serum angiopoietin-2 day 0 (ng/mL) | 23672 (13676-34468) | 18101 (11167-24166) | 0.16 |

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*Table 21* Univariate and multivariate analysis for associated factors with renal replacement therapy

| Variables | Unadjusted HR | 95% CI | p-value | Adjusted HR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| Male | 1.58 | 0.86-2.92 | 0.143 | 1.55 | 0.64-1.8 | 0.335 |
| Age | 0.96 | 0.81-1.13 | 0.587 | 0.87 | 0.68-1.12 | 0.274 |
| APACHEII | 1.28 | 0.79-2.07 | 0.32 | | | |
| SOFA | 1.12 | 1.02-1.23 | **0.027** | 1.23 | 1.05-1.43 | **0.009** |
| Nonrenal SOFA | 1.09 | 0.99-1.18 | 0.054 | | | |
| Sepsis | 2.59 | 1.27-5.28 | **0.009** | 3.87 | 1.25-11.98 | **0.019** |
| NGAL day 0 (ng/mL) | 1.03 | 0.99-1.07 | **0.079** | 1.06 | 1.01-1.12 | **0.024** |
| NT-proBNP day 0 (pg/mL) | 1.2 | 1.02-1.42 | 0.033 | 1.02 | 0.85-1.23 | 0.833 |

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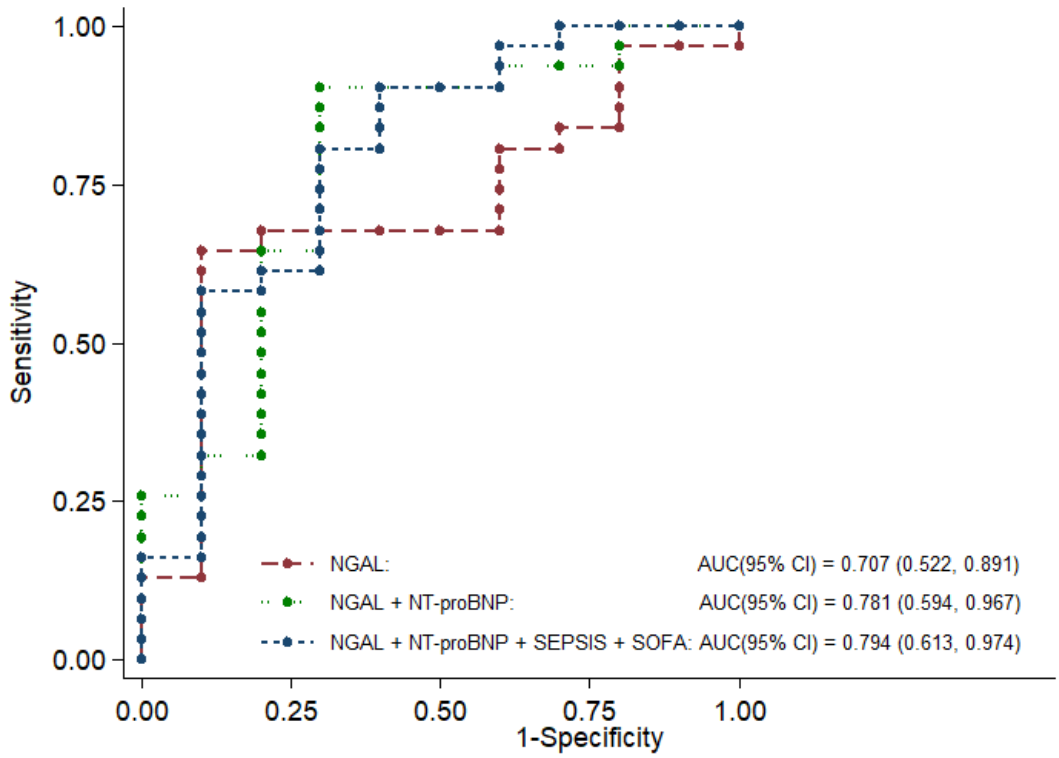
*Figure 15* AUC-ROC of baseline plasma NGAL alone, plasma NGAL plus NT-proBNP, and both biomarkers combined with clinical parameters (sepsis and SOFA score) for prediction of renal non-recovery

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# VITA

| | |
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| **AWARD RECEIVED** | Best Fellow Research Awards, the Royal College of Physicians of Thailand |

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