Effects of delayed remote ischaemic preconditioning on peri-operative myocardial and renal injury in cardiac surgery patients: A randomized clinical trial

Corresponding Author: Yunseok Jeon M.D.

Associate Professor, Department of Anaesthesiology and Pain medicine,

Seoul National University Hospital, Daehakro 101, Jongno-gu, Seoul, Korea, 110-744

Tel: 82-2-2072-2467, Fax: 82-2-747-5639

E-mail: jeonyunseok@gmail.com

Tae Kyong Kim[a], Jeong Jin Min[b], Youn Joung Cho[a], Derek J Hausenloy[c,d,e,f], Hyuk Ahn[g], Kyung-Hwan Kim[g], Ho Young Hwang[g], Deok Man Hong[a] and Yunseok Jeon[a]

[a] Department of Anaesthesiology and Pain Medicine, Seoul National University Hospital, Seoul, Korea

[b] Department of Anaesthesiology and Pain Medicine, Samsung Medical Centre, Seoul, Korea.

[c] The Hatter Cardiovascular Institute, University College London, London, UK

^d The National Institute of Health Research University College London Hospitals Biomedical Research Centre, London, UK

^e National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore, Singapore

^f Cardiovascular and Metabolic Disorders Program, Duke-National University of Singapore, Singapore, Singapore

^g Department of Thoracic and Cardiovascular Surgery, Seoul National University Hospital, Seoul, Korea

Drs. Tae Kyong Kim and Jeong Jin Min contributed equally as the first author of this manuscript.

Drs. Yunseok Jeon and Deok Man Hong contributed equally as the corresponding author of this manuscript.

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Abstract

Objectives: Remote ischaemic preconditioning (RIPC) has two time windows for organ protection: acute and delayed. Previous studies have mainly focused on the acute time window to evaluate organ protection by RIPC. We evaluated myocardial and renal protection by delayed RIPC in adult patients undergoing cardiac surgery.

Methods: A total of 160 adult patients undergoing cardiac surgery with cardiopulmonary bypass were randomized to receive either delayed RIPC (four cycles of 5 min of ischaemia followed by 5 min of reperfusion by inflation to 200 mmHg and deflation of a blood pressure cuff on the upper arm) or the control treatment 24-48 h before surgery. The primary endpoint was post-operative troponin I levels serially measured for 72 h.

Results: Post-operative troponin I values did not differ between the delayed RIPC and the control group (area under the curve for the serum troponin I at 72 h; median (IQR), 743.45 (276.36 – 1464.06) h.ng/mL and 530.78 (264.58 – 1232.61) h.ng/mL, respectively; p=0.414). Furthermore, no significant differences between groups were seen in the secondary endpoints including acute kidney injury and composite complications.

Conclusions: Delayed RIPC did not provide cardioprotective effects in patients undergoing cardiac surgery.

Key questions

What is already known about this subject?

Remote ischaemic preconditioning (RIPC) has two time windows for organ protection: acute and delayed. Previous studies have mainly focused on the acute time window to evaluate organ protection by RIPC. However, clinical trials investigating the benefits of delayed RIPC are lacking.

What does this study add?

In this randomized controlled clinical trial, patients undergoing cardiac surgery with cardiopulmonary bypass were randomized to receive either delayed RIPC or the control treatment 24-48 h before surgery. There were no significant differences in post-operative troponin I values between groups. Furthermore, no significant differences between groups were seen in the secondary endpoints including acute kidney injury and composite complications.

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How might this impact on clinical practice?

The study findings suggest that delayed RIPC may not provide cardioprotective effects in patients undergoing cardiac surgery. Further studies are needed to evaluate the systemic protective effects of delayed RIPC on various distal organs

Introduction

Remote ischaemic preconditioning (RIPC) by brief episodes of limb ischaemia and reperfusion provides protection against acute ischaemia-reperfusion injury in distal organs.[1, 2] RIPC is a non-invasive and powerful therapeutic intervention for inducing organ protection and is associated with a reduced risk of peri-operative myocardial injury after cardiac surgery.[3-7] Additionally, it provides a protective effect to other distal organs, such as the kidneys and lungs.[4, 8-10]

The protective effect of preconditioning has a biphasic pattern; acute protective effects wane after a few hours, but a delayed second window of protection occurs after 12-24 h.[11, 12] The acute effects rely on the activation of existing signaling molecules, whereas the delayed effects are achieved by increased expression of protective proteins.[11, 12] Delayed phase preconditioning provides sustained protection from myocardial infarction, as well as protective potential against myocardial stunning, arrhythmia, and endothelial dysfunction.[11]

In contrary to previous studies that demonstrated cardioprotective effect of RIPC,[1, 2] recent large clinical trials in cardiac surgery have failed to show clinical benefit by acute RIPC.[13, 14] Although we do not know which factors interfere with the protective effect of acute RIPC in cardiac surgery, the different time window of ischaemia in delayed RIPC may have the advantage to bypass the unknown interfering factors. However, unlike acute RIPC, clinical trials investigating the benefits of delayed RIPC are lacking. We hypothesized that delayed RIPC has clinically significant myocardial protective effects. The aim of the study

was to investigate whether delayed RIPC decreased myocardial and renal injury in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB).

Methods

Ethical approval for this study (1211-041-441) was provided by the institutional review board of Seoul National University Hospital. The study protocol was registered at ClinicalTrials.gov (NCT01903161). Written informed consent was obtained from all patients enrolled in the study. There were no important changes to the methods or outcomes after trial commencement. Patients aged 18 to 80 years and scheduled for elective cardiac surgery with CPB were included. Exclusion criteria were as follows: left ventricular ejection fraction < 30%, pre-operative administration of vasopressors or inotropes, chronic liver disease with Child-Pugh class C, chronic kidney disease requiring dialysis, diabetes, peripheral vascular disease affecting the upper limbs, descending thoracic aortic surgery, and rare surgeries, such as cardiac transplantation or correction of congenital anomalies. A total of 160 patients were included in the study from May 2013 to January 2015.

Randomization

This was a single-center, parallel-group randomized study conducted at the Seoul National University Hospital, a tertiary hospital in Seoul, Korea. Eligible patients were randomly allocated to either the delayed RIPC group or the control group using a computer-generated list. The randomization sequence was created with a 1:1 allocation using a random block size of 4. The random list was generated by a statistician who was not involved in the study and who was blinded to all patients, medical personnel, and investigators.

Remote ischaemic preconditioning

An independent nurse performed RIPC 24 to 48 h prior to surgery. RIPC consisted of four

cycles of 5 min of ischaemia, which was induced by a blood pressure cuff in the upper arm inflated to 200 mmHg, followed by 5 min of reperfusion, during which the cuff was deflated. In the control group, the same blood pressure cuff was placed around the upper arm, but the cuff was inflated to 10 mmHg and ischaemic preconditioning was not induced.

Anaesthesia and cardiopulmonary bypass techniques

All patients received standard peri-operative care. Routine monitoring included a bispectral index, cerebral oximetry, a pulmonary artery catheter, and transoesophageal echocardiography. Anaesthesia was induced with intravenous midazolam 0.15 mg/kg, sufentanil 1 µg/kg, and vecuronium 0.15 mg/kg, and was maintained with target controlled infusions of remifentanil 6-12 ng/mL and propofol 1.5-2.5 µg/mL, maintaining bispectral index values between 40 and 60.

Study patients underwent cardiac surgery using a non-pulsatile CPB technique with a membrane oxygenator and cardiotomy suction. Cardiac protection was achieved using antegrade or retrograde cold-blood cardioplegia. Heparin was administered before CPB and was reversed by protamine after discontinuing CPB. The target activated clotting times during surgery were more than 500 s. At the end of surgery, patients were transferred to the intensive care unit. Intensive care unit management was provided by attending physicians and standardized for all patients according to the routine protocol of our institution.

Study outcomes

The primary endpoint was serum troponin I, measured at 1, 6, 12, 24, 48, and 72 h post-operatively. Serum troponin I has previously been used as a marker of peri-operative

myocardial injury after cardiac surgery.[3, 4, 15] The secondary endpoints included post-operative serum creatinine levels, acute kidney injury (AKI), defined by the Acute Kidney Injury Network (AKIN) staging system,[16] and composite complications. In the AKIN criteria, serum creatinine criteria was used and the pre-operative creatinine levels were used as baseline levels. Composite complications included in-hospital death, myocardial infarction, new onset atrial fibrillation, stroke, AKI, respiratory failure, persistent cardiogenic shock, and gastrointestinal complications.[17] Myocardial infarction was defined as an elevation of cardiac biomarker values ($> 10\times$ 99th percentile upper reference limit) in patients with normal baseline troponin values (< 99th percentile upper reference limit). Additionally, new pathological Q waves or new left bundle branch block, or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, was required.[18] Respiratory failure was defined as the need for post-operative mechanical ventilation for > 72 h. Persistent cardiogenic shock was defined as use of inotropic agents, vasopressors, or a mechanical assist device for more than 72 h. Gastrointestinal complications were defined as gastrointestinal bleeding requiring transfusion, pancreatitis requiring nasogastric suction, cholecystitis requiring drainage, or mesenteric ischaemia requiring exploration. Additional secondary end points were cardiovascular mortality, ventricular arrhythmia, renal replacement therapy, mechanical ventilation time, intensive care unit and hospital length of stay, use of intraaortic balloon pump or extracorporeal membrane oxygenation, reoperation for bleeding, and post-operative delirium.

Statistical analysis

In the study by Hong et al.,[15] the area under the curve (AUC) of post-operative troponin I was 69.4 ± 74.5 h·ng/mL. Presuming that the difference of 50% in troponin I AUC was clinically significant, 74 patients were required in each group to detect a difference, with a type I error of 0.05 and a power of 0.8. To allow for dropouts, 80 patients were recruited for each group. Continuous variables of patient demographics and group characteristics were compared using the Student's t-test or the Mann–Whitney U test after testing for normality. Categorical variables were analyzed using the χ^2 test or Fisher's exact test, where appropriate. Changes in serum troponin I and creatinine over time were analyzed using repeated measures analysis of variance (ANOVA).. The AUC was determined using the standard trapezoidal method. A $p<0.05$ was regarded as statistically significant. Adjusted p values were calculated using Hochberg method in order to correct for multiple testing. Data were analyzed using SPSS ver. 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients

Of the 232 patients screened, 72 were excluded; 16 for left ventricular ejection fraction < 30%, 18 for renal impairment requiring renal replacement therapy, 19 for diabetes, 9 for pre-operative administration of vasopressors or inotropes, 8 for peripheral vascular disease, and 2 for declining to participate (Fig. 1). One hundred sixty randomized patients received the allocated interventions and were included in the final analysis. Demographic data for the patients are shown in Table 1. Time from RIPC or sham to aorta cross clamp and reperfusion, aortic cross-clamp time, duration of CPB, and surgery were comparable between groups (Table 2).

Serum troponin I

Serum troponin I levels significantly increased after surgery and peaked 1 h post-operatively (Fig. 2). Changes in serum troponin I were not significantly different between groups (p=0.662, Fig. 2). Moreover, the total 72 h AUC of troponin I did not differ between the delayed RIPC and control groups (median (IQR), 743.45 (276.36 – 1464.06) h.ng/mL vs. 530.78 (264.58 – 1232.61) h.ng/mL, p=0.414).

Acute kidney injury

The incidence of post-operative AKI based on the AKIN staging system was decreased in the delayed RIPC group compared to the control group (30.0% vs. 47.5%; RR, 0.632; 95% CI, 0.421 – 0.948; p=0.023). However, adjusted p value for multiple comparison was not

statistically significant (RR, 0.632; 99.8% CI 0.338 – 1.232; p=0.46, Table 3). In both groups, most AKIs were categorized as AKIN class 1. The number of patients categorized as AKIN class 3, which includes individuals who received renal replacement therapy, was comparable between groups (5.0% vs. 5.0%, see Supplementary dada). Changes in serum creatinine were not significantly different between groups (p=0.714, Fig. 3).

Composite complications

The rate of composite complications was lower in the delayed RIPC group compared to the control group (65.0% vs. 81.3%; RR, 0.800; 95% CI, 0.660 – 0.970; p=0.020), but not significant when adjusted for multiple comparisons (RR, 0.800; 99.8% CI 0.574 – 1.091; adjusted p=0.42, Table 3). In-hospital mortality rate and cardiovascular mortality rate were higher in the control group but the difference did not reach statistical significance (Table 3). Causes of death included cardiogenic shock (four patients in the control group and one patient in the delayed RIPC group), diffuse bleeding of unknown etiology (one patient in the control group), and septic shock (one patient in the delayed RIPC group). Incidence of post-operative new onset atrial fibrillation, risks of myocardial infarction, respiratory failure, and gastrointestinal complications were lower in the delayed RIPC group but the difference did not reach statistical significance.

Discussion

In patients undergoing cardiac surgery with CPB, delayed RIPC did not show cardioprotective effects as assessed by troponin I levels. Cardiac surgery with CPB can cause global myocardial ischaemia-reperfusion injury, the presence of which can be quantified by measuring cardiac enzymes and is associated with worse clinical outcomes. RIPC is a non-invasive, inexpensive, and powerful therapeutic intervention for inducing cardioprotection in patients undergoing cardiac surgery; previous studies have shown that RIPC reduces peri-operative myocardial injury and possibly improves prognosis.[4, 5, 7] However, the clinical effects of delayed RIPC have not been adequately studied. Wagner et al. first reported that the delayed phase of RIPC could reduce peri-operative myocardial injury in cardiac surgery.[19] However, this effect was not evident in the study by Pavione et al., which was performed in children undergoing CPB.[20]

Contrary to our study, previous meta-analyses of clinical trial data on acute RIPC showed a myocardial protective effect,[6, 7] which could be due to various possibilities. First, acute RIPC may be more effective than delayed RIPC for myocardial protection. In a previous animal study, only acute RIPC decreased reperfusion-induced ventricular arrhythmias.[21] However, we did not compare early and delayed RIPC in this study. Second, previous acute RIPC studies with positive results were mostly performed in patients undergoing coronary artery bypass graft or uncomplicated single valve surgery,[4, 6, 15] while in this study, 58 patients underwent complicated cardiac surgeries such as double or triple valve surgery, and aorta cross clamping time was much longer than in previous studies.[3-5] These findings raise the possibility that the myocardial protective effects of RIPC might be insufficient in

complicated cardiac surgeries with significant myocardial injury.[22]

The prevalence of AKIN class 1 AKI was less in the delayed RIPC group compared to the control group in this study. It is well-known that AKI is frequent after cardiac surgery and is associated with morbidity and mortality.[23] Previous meta-analyses reported that there is no definitive evidence of renal protection after RIPC.[6, 7] However, several recent studies have demonstrated the beneficial role of acute phase RIPC on kidney protection.[8, 10] Interestingly, in a meta-analysis investigating RIPC in animal models, delayed RIPC was more effective than acute RIPC in reducing serum creatinine after renal ischaemia-reperfusion injury (standardized mean difference 2.43; 95% confidence interval, 1.29 – 3.57).[24] In a recent study in pigs, delayed RIPC showed significant reduction in renal injury biomarkers such as urinary neutrophil gelatinase-associated lipocalin, and kidney injury molecule-1 compared to acute RIPC.[25] Considering that even a small increase in serum creatinine levels is associated with poor outcomes,[23] our study suggests that delayed RIPC might be a therapeutic option to attenuate AKI. However, our study was not powered to detect the difference in AKI, and the finding should be interpreted with caution.

It has been demonstrated that RIPC has systemic protective effects on various distal organs;[2] however, in our previous clinical trial on 1280 cardiac surgery patients, acute RIPC did not decrease composite complications.[17] Recent multicenter trials, The Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Graft Surgery (ERICCA) trial[13] and the Remote Ischemic Preconditioning for Heart Surgery (RIPHeart) Study,[14] also failed to show a relevant clinical benefits of acute RIPC in cardiac surgery. The authors suggested that lack of standardization of

perioperative anaesthesia may have affected the efficacy of RIPC. Then, delayed RIPC 24 to 48 h prior to the cardiac surgery may be more practical in the clinical setting compared with the acute RIPC which may be affected by multiple confounders such as propofol during the surgery. Also, we hypothesize that delayed RIPC does not result in the transient harmful effects of RIPC techniques leading to inflammation and coagulation;[26, 27] if true, this suggests that delayed RIPC may be more beneficial in cardiac surgery patients compared with acute RIPC. Although not significant after adjustment, the rate of composite complications was lower in the delayed RIPC group than in the control group in this study. Similar trends were observed for in-hospital mortality, cardiovascular mortality, myocardial infarction and new-onset atrial fibrillation.

The exact mechanisms underlying the organ protective signal transfer from remote ischaemic stimuli to distal organs are not yet clear; however, neuronal and humoral transmission are widely suggested.[2] Time-dependent transcription and synthesis of cardioprotective mediators or neuronal release of a signal molecule may account for the two distinct windows of organ protection in RIPC.[2, 12] Unlike acute RIPC, delayed RIPC requires the synthesis of new proteins such as nitric oxide synthase, cyclooxygenase-2, aldose reductase, and antioxidant enzymes.[11, 12] Through its systemic effects, delayed RIPC may provide distal organ protection, such as kidney protection, as suggested in this study. While the acute preconditioning effect lasts only 2-3 h, delayed preconditioning has a longer duration of protection, ranging from 3-4 days.[11, 12] It is not always easy in cardiac surgeries even in elective cases that ischemia-reperfusion injury occur on the exact protective time window. It may be one of the reason for the inconsistent results of RIPC in previous

clinical studies. The longer time window of delayed preconditioning may be more beneficial in a real clinical setting, such as during long aorta cross clamp durations in complex cardiac surgeries in which peri-operative ischaemic insults do not always occur within 2-3 h after preconditioning.

This study had several limitations. First, study was not powered to detect AKI or composite outcomes, and the results should be interpreted with caution. Second, confounders such as patient age, sex, comorbidities, and drugs may have affected the study outcomes. In this study, patients with diabetes were excluded since release of a humoral cardioprotective factor is attenuated in diabetic patients.[28] Beta blockers are known to inhibit preconditioning pathways;[28] however, use of beta blockers were comparable between groups. Third, the choice of anaesthetics can be a major confounder in surgical settings. We avoided volatile anaesthetics because inherent preconditioning might be fully exploited by a volatile anaesthetic itself.[29] Kottenberg et al. reported that propofol may interfere with the cardioprotective effects of RIPC.[30] However, several studies reported a significant decrease in myocardial injury following RIPC under propofol anaesthesia.[5, 9, 15] Thus, more evidence is needed to clarify the effects of propofol on RIPC effects. Moreover, larger multicenter randomized clinical trials are required to fully elucidate the effects of delayed RIPC on clinical outcomes.

In summary, delayed RIPC did not provide cardioprotective effects in patients undergoing cardiac surgery with CPB.

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Table 1. Baseline characteristics

	Delayed RIPC (n=80)	Control (n=80)	p Value
Age, years	61.8±11.1	62.8±13.2	0.618
Male sex, n (%)	39 (48.8)	46 (57.5)	0.267
Weight, kg	58.0±10.4	59.3±8.9	0.393
Height, cm	160.0±9.7	162.6±10.0	0.102
Body mass index, kg/cm^2	22.5±3.0	22.4±2.8	0.789
Hypertension, n (%)	24 (30.0)	31 (38.8)	0.244
Previous stroke, n (%)	6 (7.5)	8 (10.0)	0.576
Current smoker, n (%)	13 (16.3)	15 (18.8)	0.677
Left ventricle ejection fraction, %	59.2±8.9	57.7±9.8	0.335
Congestive heart failure, n (%)	13 (16.3)	11 (13.8)	0.658
Previous cardiac surgery, n (%)	21 (26.3)	15 (18.8)	0.256
EuroSCORE II	2.7±2.6	2.4±1.9	0.458
Serum troponin I, ng/mL	0.0±0.0	0.8±6.1	0.316

Serum creatinine, mg/dL	0.9±0.2	0.9±0.3	0.684
Serum lactate, mmol/L	1.1±0.4	1.1±0.5	0.490
Platelet count, x10^9/L	197.9±57.9	200.0±65.5	0.886
Fibrinogen, mg/dL	279.0±69.5	296.4±56.1	0.056
Type of procedures, n (%)			
Mitral valve (alone)	21 (26.3)	14 (17.5)	0.181
Aortic valve (alone)	22 (27.5)	22 (27.5)	1.000
Other valve (alone)	4 (5.0)	4 (5.0)	1.000
Aorta surgery (alone)	2 (2.5)	4 (5.0)	0.681
Other procedures (alone)	4 (5.0)	5 (6.3)	0.732
Combined procedures	27 (33.8)	31 (38.8)	0.511
Coronary artery bypass graft	5 (6.3)	5 (6.3)	1.000

Continuous data are reported as means ± SD. RIPC, remote ischaemic preconditioning;

EuroSCORE II European System for Cardiac Operative Risk Evaluation II.

Table 2. Intra-operative characteristics

	Delayed RIPC (n=80)	Control (n=80)	p Value
Time from RIPC or sham to aorta cross clamp, h	29.5±5.8	29.3±6.9	0.837
Time from RIPC or sham to reperfusion, h	31.9±5.9	31.8±6.7	0.923
Aortic cross-clamp duration, min	145.2±59.0	149.2±58.7	0.663
Cardiopulmonary bypass duration, min	228.4±85.8	233.4±79.2	0.707
Duration of surgery, min	447.4±142.2	445.7±134.8	0.937

Data are presented as means ± SD. RIPC, remote ischaemic preconditioning

Table 3. Clinical outcomes

	Delayed RIPC (n=80)	Control (n=80)	RR (95% CI)	p Value	RR (adjusted CI)	Adjusted p Value[c]
Mechanical ventilation time, h	20 (15–46)	20 (16–46)		0.877		NS
ICU length of stay, day	4 (3–7)	4 (3–8)		0.525		NS
Hospital length of stay, day	14 (9–20)	14 (10–20)		0.874		NS
Use of IABP or ECMO, n (%)	8 (10.0)	10 (12.5)	0.800 (0.333–1.922)	0.617	0.800 (0.131–2.875)	NS
Reoperation for bleeding, n (%)	4 (5.0)	6 (7.5)	0.667 (0.196–2.273)	0.514	0.667 (0.086–9.785)	NS
In-hospital mortality, n (%)	2 (2.5)	5 (6.3)	0.400 (0.080–2.002)	0.246	0.400 (0.091–10.001)	NS
Cardiovascular mortality, n (%)	1 (1.3%)	4 (5.0%)	0.250 (0.029–2.188)	0.367	NA	NS
Myocardial infarction, n (%)	0 (0.0)	3 (3.8)	NA	0.080	NA	NS

New-onset atrial fibrillation, n (%)	27 (33.8)	39 (48.8)	0.692 (0.473–1.013)	0.054	0.692 (0.294–1.283)	NS
Ventricular arrhythmia, n (%)	21 (26.3)	21 (26.3)	1.000 (0.595–1.681)	1.000	1.000 (0.393–2.260)	NS
Stroke, n (%)	1 (1.3)	1 (1.3)	1.000 (0.064–15.712)	1.000	NA	NS
Post-operative delirium, n (%)	18 (22.5)	17 (21.3)	1.059 (0.589–1.902)	0.848	1.059 (0.367–3.206)	NS
Acute kidney injury, n (%)	24 (30.0)	38 (47.5)	0.632 (0.421–0.948)	0.023	0.632 (0.338–1.232)	0.46
AKIN 1, n (%)	15 (18.8)	33 (41.3)				
AKIN 2, n (%)	5 (6.3)	1 (1.3)				
AKIN 3[a], n (%)	4 (5.0)	4 (5.0)				
RRT within 48 h, n (%)	2 (2.5)	2 (2.5)	1.000 (0.144–6.926)	1.000	NA	NS
RRT in-hospital, n (%)	6 (7.5)	6 (7.5)	1.000 (0.337–2.969)	1.000	1.000 (0.098–6.701)	NS
Respiratory failure, n (%)	6 (7.5)	10 (12.5)	0.600 (0.229–1.573)	0.292	0.600 (0.076–6.310)	NS

Persistent cardiogenic shock, n (%)	14 (17.5)	14 (17.5)	1.000 (0.510–1.960)	1.000	1.000 (0.313-3.678)	NS
Gastrointestinal complications, n (%)	2 (2.5)	3 (3.8)	0.667 (0.114–3.883)	0.650	NA	NS
Composite complications[b], n (%)	52 (65.0)	65 (81.3)	0.800 (0.660–0.970)	0.020	0.800 (0.574-1.091)	0.42

Continuous data are presented as means ± SD, or median (IQR). [a]includes RRT, [b]Composite complications include in-hospital death, myocardial infarction, new onset atrial fibrillation, stroke, acute kidney injury, respiratory failure, and persistent cardiogenic shock, and gastrointestinal complication, [c]Adjusted for multiple comparisons using the Hochberg procedure. NS, adjusted p value>0.999. CI, confidence interval; RR, relative risk; RIPC, remote ischaemic preconditioning; ICU, intensive care unit; IABP, intraaortic balloon pump; ECMO, extracorporeal membrane oxygenation; AKIN, Acute Kidney Injury Network; RRT, renal replacement therapy; NA, not applicable.

Figure legends

Figure 1. Consort diagram.

Figure 2. Peri-operative concentrations of serum troponin I.

Data are presented as the median and quartiles. Error bars indicate the 90th and 10th percentiles. Asterisks indicate significant changes compared with the pre-operative value (*$p<0.05$). RIPC, remote ischaemic preconditioning.

Figure 3. Peri-operative concentrations of serum creatinine.

Data are presented as the median and quartiles. Error bars indicate the 90th and 10th percentiles. Asterisks indicate significant changes compared with the pre-operative value (*$p<0.05$). RIPC, remote ischaemic preconditioning; POD, post-operative day.

Enrollment

Assessed for eligibility (*n* = 232)

Excluded (*n* = 72)
  ˙ Left ventricular ejection fraction < 30% (*n* = 16)
  ˙ Renal impairment requiring renal replacement
  therapy (*n* = 18)
  ˙ Diabetes (*n* = 19)
  ˙ Preoperative administration of vasopressors or
  inotropes (*n* = 9)
  ˙ Peripheral vascular disease (*n* = 8)
  ˙ Declined to participate (*n* = 2)

Randomized (*n* = 160)

Allocation

Allocated to delayed RIPC group (*n* = 80)
  ˙ Received allocated intervention (*n* = 80)
  ˙ Did not receive allocated intervention (*n* = 0)

Allocated to control group (*n* = 80)
  ˙ Received allocated intervention (*n* = 80)
  ˙ Did not receive allocated intervention (*n* = 0)

Follow-Up

Lost to follow-up (*n* = 0)
Discontinued intervention (*n* = 0)

Lost to follow-up (*n* = 0)
Discontinued intervention (*n* = 0)

Analysis

Analyzed (*n* = 80)
  ˙ Excluded from analysis (*n* = 0)

Analyzed (*n* = 80)
  ˙ Excluded from analysis (*n* = 0)