EDITORIAL

Remote Ischemic Preconditioning for Kidney Protection

Jenny Szu-Chin Pan, MD; David Sheikh-Hamad, MD

Acute kidney injury (AKI) is common and frequently associated with substantial morbidity and mortality. Elevated serum creatinine levels in patients after surgery are associated with poor outcomes.[1,2] A small increase in serum creatinine in hospitalized patients is associated with higher mortality, longer hospitalization, and higher cost of care.[3] Acute tubular necrosis (ATN), defined as acute injury to the renal tubular epithelial cells, may occur following severe renal ischemia or exposure to nephrotoxins.

Preconditioning-mediated protection is a phenomenon in which tissue, once exposed to a specific type of insult, will be protected from injury during a repeated similar or sometimes dissimilar insult.[4] The protective phenotype (preconditioning) in response to ischemia depends on a coordinated response at the genomic, molecular, cellular, and tissue levels, in what is described as "genomic reprogramming."[5] Preconditioning-mediated protection is well described for the brain and heart, and recent reports suggest that it also functions for the kidney in ATN. For instance, in experimental mouse/rat models of ischemia/reperfusion kidney injury, in which bilateral kidney pedicles (arteries and veins) are clamped for 30 to 45 minutes, AKI develops within 24 hours; invariably, kidney function recovers within a week. However, if the kidneys are subjected to an equivalent insult 1 or 2 weeks after the initial insult, an equal increase in serum creatinine or kidney inflammation markers compared with the initial insult is not observed.[6] Resistance to repeated ischemic episodes afforded by 30 minutes of bilateral kidney ischemia can persist for 12 weeks after preconditioning,[6,7] whereas a shorter period of ischemia (15 minutes) is partially protective against subsequent ischemic injury imposed 8 days later. Similarly, the kidneys may display attenuated ischemia/reperfusion injury following nephrotoxic (eg, cisplatin) injury, and vice versa.[8] A major limitation to the clinical use of this ischemic preconditioning strategy is the need for direct access to the blood supply of the organ at risk.

Related article

Recent data suggest that ischemia in one organ is accompanied with protective changes in distant organs, a phenomenon known as remote ischemic preconditioning (RIPC). Application of one or more brief cycles of nonlethal ischemia/reperfusion to an organ or tissue may protect a remote organ or tissue from a sustained episode of lethal ischemia/reperfusion. The beneficial effects of RIPC have been demonstrated in diverse organs and tissues (lung, liver, kidney, intestine, brain, skeletal muscle) subjected to acute ischemia/reperfusion,[9] and the discovery that RIPC can be induced noninvasively by simple inflation and deflation of a standard blood pressure cuff placed on a limb has facilitated its translation into the clinical setting.

The report by Zarbock and colleagues in this issue of *JAMA*[10] examined the effects of RIPC on the rate and severity of AKI in patients undergoing cardiac surgery. In a multicenter trial, 240 patients at high risk for AKI (Cleveland Clinic Foundation score ≥6) were randomized to receive either RIPC (n = 120) or sham control (n = 120) after induction of anesthesia. The RIPC involved 3 cycles of 5 minutes of ischemia induced by inflation of a blood pressure cuff to 200 mm Hg or at least 50 mm greater than systolic blood pressure in one upper arm, followed by 5 minutes of reperfusion with the cuff deflated. The sham RIPC (control) involved 3 cycles of "pseudoischemia," consisting of 5 minutes of blood pressure cuff inflation to 20 mm Hg, followed by 5 minutes of cuff deflation.

All patients completed follow-up 30 days after surgery and were analyzed according to the intention-to-treat principle. The primary end point was the rate of AKI defined by KDIGO criteria within the first 72 hours after cardiac surgery.[11] Secondary end points included use of renal replacement therapy (RRT), duration of intensive care, occurrence of myocardial infarction and stroke, in-hospital and 30-day mortality, and change in AKI biomarkers. Acute kidney injury was significantly reduced with RIPC (45/120; 37.5%) compared with control (63/120; 52.5%), with an absolute risk reduction (ARR) of 15%. Fewer patients received RRT after RIPC (7 [5.8%] vs 19 control [15.8%]; ARR, 10%) and RIPC reduced intensive care unit stay (3 vs 4 days). RIPC had no significant effect on myocardial infarction, stroke, or mortality.

Even though the mechanism(s) underlying preconditioning remains elusive, a few clues about its nature have emerged. Upregulation of inducible nitric oxide synthase and heat shock proteins[7,12] as well as activation of the endoplasmic reticulum stress response[13] may play a role in the longer-term kidney protection attributed to ischemic preconditioning. Recent data suggest that recruitment of mesenchymal stem cells is critical for this protection. Regeneration of proximal tubule epithelium after ischemic injury appears to be intrinsic to the kidney and does not require transdifferentiation of bone marrow–derived stem cells.[14]

However, bone marrow–derived leukocytes and cells bearing endothelial markers (mesenchymal cells) are detected in the kidney interstitium after ischemic injury.[14,15] Kuo et al[16] suggested that injured kidney cells (endothelial) recruit mesenchymal stem cells through the release of preformed vesicles (Weibel-Palade bodies) packed with messenger signals that include IL-8, eotaxin 3, von Willebrand factor, and angiopoietin 2. A trigger for the release of these vesicles is uric acid, a product of xanthine oxidase activation in the ischemic organ, through interaction with Toll-like receptors TLR2 and TLR4. Angiopoietin 2 amplifies release of Weibel-Palade bodies and plays a pivotal role in the recruitment of mesenchymal stem cells in response to uric acid and potentially other "alarm signals."[16] Administration of mesenchymal stem cells to rats protects against ischemic kidney injury, and this protection is associated with up-

regulation of anti-inflammatory cytokines and down-regulation of proinflammatory cytokines.[17]

The recruitment of mesenchymal stem cells following injury is not unique to the kidney: the lungs attract mesenchymal stem cells in response to bleomycin-induced injury.[18] In this context, the protection afforded by mesenchymal stem cells appears to be mediated through release of the mitochondrially targeted antioxidant and prosurvival factor stanniocalcin 1,[18] which plays an important role in neuronal and cardiac protection after preconditioning.[19,20] Thus, the recruitment of mesenchymal stem cells in response to ischemic injury promotes recovery of the kidney after ischemia/reperfusion and may underlie the preconditioning phenomenon.

RIPC may release damage-associated molecular patterns molecules that interact with TLRs on proximal tubule cells to induce natural defenses that protect the kidney from subsequent inflammatory or ischemic stress.[21] In the study by Zarbock et al,[10] RIPC increased urinary high mobility group box (HMGB) 1, an endogenous factor that mediates activation of the innate immune response including chemotaxis and proinflammatory cytokine release. For cardiac protection, transient limb ischemia releases a low-molecular-weight (<15-kDa) factor that protects the myocardium against ischemia/reperfusion injury in a mechanism that requires opioid-receptor activation and modification of mitochondrial function via ATP-sensitive potassium channels.[9] However, it is not clear whether RIPC is mediated through dispatch of mesenchymal stem cells to various organs after the ischemic episodes in the arm or leg muscle.

Therapeutic strategies to protect against ischemic kidney injury and improve patient outcomes are lacking.[22] RIPC, as demonstrated in the study by Zarbock and colleagues, may offer a novel inexpensive and noninvasive clinical intervention to reduce the occurrence and severity of AKI. Further studies are needed to determine whether a longer duration of limb ischemia or earlier induction of RIPC confers better renoprotection; 37.5% of the patients in the RIPC group still developed AKI. Experimental animal models of RIPC induce early (immediate to 4 hours) and late (24-72 hours) biphasic protection against ischemic injury.[23]

Before RIPC is adopted for clinical use, the potential risks and adverse effects must be considered carefully. For example, prolonged unilateral kidney ischemia leads to changes in the contralateral kidney and heart.[24] Cardiac changes after experimental renal ischemia include cytokine induction, leukocyte infiltration, cell apoptosis, and impaired cardiac function. While remote kidney/cardiac preconditioning after limb muscle ischemia may differ from the changes observed in the heart after kidney ischemia, effects of repeated limb ischemia with RIPC are not known and clinicians should be mindful of potential harms before adopting this approach widely.

ARTICLE INFORMATION

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Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Effect of Remote Ischemic Preconditioning on Kidney Injury Among High-Risk Patients Undergoing Cardiac Surgery
A Randomized Clinical Trial

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IMPORTANCE No interventions have yet been identified to reduce the risk of acute kidney injury in the setting of cardiac surgery.

OBJECTIVE To determine whether remote ischemic preconditioning reduces the rate and severity of acute kidney injury in patients undergoing cardiac surgery.

DESIGN, SETTING, AND PARTICIPANTS In this multicenter trial, we enrolled 240 patients at high risk for acute kidney injury, as identified by a Cleveland Clinic Foundation score of 6 or higher, between August 2013 and June 2014 at 4 hospitals in Germany. We randomized them to receive remote ischemic preconditioning or sham remote ischemic preconditioning (control). All patients completed follow-up 30 days after surgery and were analyzed according to the intention-to-treat principle.

INTERVENTIONS Patients received either remote ischemic preconditioning (3 cycles of 5-minute ischemia and 5-minute reperfusion in one upper arm after induction of anesthesia) or sham remote ischemic preconditioning (control), both via blood pressure cuff inflation.

MAIN OUTCOMES AND MEASURES The primary end point was the rate of acute kidney injury defined by Kidney Disease: Improving Global Outcomes criteria within the first 72 hours after cardiac surgery. Secondary end points included use of renal replacement therapy, duration of intensive care unit stay, occurrence of myocardial infarction and stroke, in-hospital and 30-day mortality, and change in acute kidney injury biomarkers.

RESULTS Acute kidney injury was significantly reduced with remote ischemic preconditioning (45 of 120 patients [37.5%]) compared with control (63 of 120 patients [52.5%]; absolute risk reduction, 15%; 95% CI, 2.56%-27.44%; $P = .02$). Fewer patients receiving remote ischemic preconditioning received renal replacement therapy (7 [5.8%] vs 19 [15.8%]; absolute risk reduction, 10%; 95% CI, 2.25%-17.75%; $P = .01$), and remote ischemic preconditioning reduced intensive care unit stay (3 days [interquartile range, 2-5]) vs 4 days (interquartile range, 2-7) ($P = .04$). There was no significant effect of remote ischemic preconditioning on myocardial infarction, stroke, or mortality. Remote ischemic preconditioning significantly attenuated the release of urinary insulinlike growth factor–binding protein 7 and tissue inhibitor of metalloproteinases 2 after surgery (remote ischemic preconditioning, 0.36 vs control, 0.97 ng/mL^2/1000; difference, 0.61; 95% CI, 0.27-0.86; $P < .001$). No adverse events were reported with remote ischemic preconditioning.

CONCLUSIONS AND RELEVANCE Among high-risk patients undergoing cardiac surgery, remote ischemic preconditioning compared with no ischemic preconditioning significantly reduced the rate of acute kidney injury and use of renal replacement therapy. The observed reduction in the rate of acute kidney injury and the need for renal replacement warrants further investigation.

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Editorial

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Acute kidney injury is a well-recognized complication after cardiac surgery and significantly affects morbidity and mortality.[1,2] Up to 30% of patients develop acute kidney injury after cardiac surgery, whereas severe acute kidney injury requiring dialysis is relatively rare.[3] Approximately 1% of all patients undergoing cardiac surgery develop a severe dialysis-dependent acute kidney injury, and this severity of injury is associated with especially poor outcomes.[1] Although the mechanisms of acute kidney injury are not fully understood, injury to renal tubular epithelial cells is a universal aspect of the disease. Despite numerous clinical trials using several interventions,[4] a reliable means to prevent acute kidney injury remains elusive.

HMGB *high-mobility group box*

IGFBP7 *insulinlike growth factor–binding protein 7*

NGAL *neutrophil gelatinase-associated lipocalin*

TIMP-2 *tissue inhibitor of metalloproteinases 2*

Remote ischemic preconditioning elicited by brief episodes of ischemia and reperfusion in distant tissue may provide protection from subsequent injury.[5] In cardiac surgery, adverse outcomes are mainly linked to perioperative myocardial injury.[6]

Remote ischemic preconditioning may attenuate renal injury by releasing various molecules such as damage-associated molecular patterns that are then filtered by the kidney and signal through Toll-like receptors in the proximal tubule epithelia.[5,7] This signaling may then induce natural defenses such as bioenergetic down-regulation and temporary cell-cycle arrest.[8] These defenses, once engaged, can then protect the kidney during subsequent inflammatory or ischemic stress.

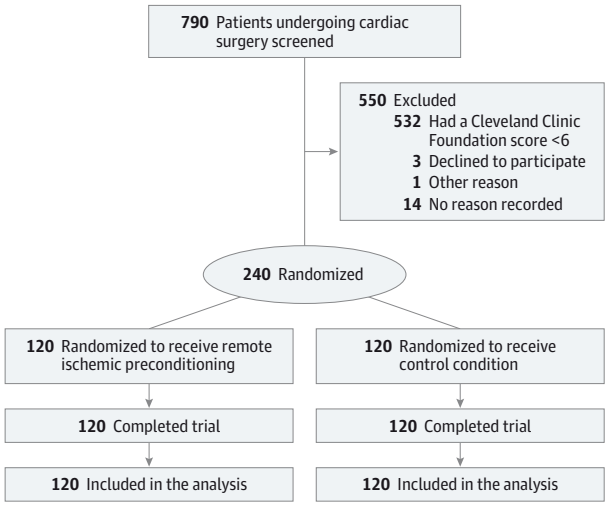
However, despite this rationale, 3 small single-center randomized trials investigating the effect of remote ischemic preconditioning on acute kidney injury after cardiac surgery have shown conflicting results.[9-11] Thus, a large randomized study with a robust and relevant clinical end point has been called for.[12] As an initial step to achieving this goal, we performed a multicenter, randomized, controlled, clinical trial to investigate whether remote ischemic preconditioning could reduce the occurrence and severity of acute kidney injury as defined by Kidney Disease: Improving Global Outcomes criteria[13] and to analyze other relevant clinical outcomes of remote ischemic preconditioning in cardiac surgery patients at high risk for acute kidney injury after on-pump cardiac surgery. Our goal was to acquire phase 2 equivalent data to support a larger multicenter trial.

Methods

Study Design and Participants

After obtaining approval from the institutional review boards at each site, we performed a multicenter, double-blind, randomized clinical trial (study protocol appears in Supplement 1). Consecutive patients were approached for enrollment during preadmission consultations and provided written informed consent. The study was conducted according to the principles of the Declaration of Helsinki. Eligible patients were adults at high risk for acute kidney injury who underwent cardiac surgery with the use of cardiopulmonary bypass at the universities of Münster, Tübingen, Freiburg, or Bochum (all in Germany) between August 2013 and June 2014. A Cleveland Clinic Foundation score (eTable 1 in Supplement 2) of 6 or higher was used to define patients at high risk for acute kidney injury.[14] The score is composed of different risk factors, including patient characteristics, comorbidities, and type of surgery.[14] Exclusion criteria were acute myocardial infarction up to 7 days before surgery, age younger than 18 years, off-pump heart surgery, preexisting acute kidney injury, kidney transplantation, chronic kidney disease with a glomerular filtration rate less than 30 mL/min, pregnancy, peripheral vascular disease affecting the upper limbs, hepatorenal syndrome, and drug therapy with sulfonamide or nicorandil (preconditioning-blocking and preconditioning-mimetic medication, respectively).

Figure 1. Participant Flow of Remote Ischemic Preconditioning in Patients Undergoing Cardiac Surgery



Randomization and Blinding

Patients were randomized on a 1:1 basis, stratified by center. Randomization codes were computer generated and concealed from investigators. On the day of surgery, patients were assigned to undergo either remote ischemic preconditioning or sham remote ischemic preconditioning (control) (**Figure 1**), and the intervention was provided by an investigator not involved in the care of the patient. Patients, anesthesiologists, staff providing care of the patient, cardiac surgeons, and intensive care physicians were unaware of treatment assignment.

Procedures

Anesthesia was induced according to the standard of care at each center and maintained with volatile anesthetics because propofol may interfere with remote ischemic preconditioning.[15] According to a recently published review,[16] we standardized the management of cardiopulmonary bypass as follows: mean arterial blood pressure of 60 to 70 mm Hg, the use of nonpulsatile cardiopulmonary bypass,

α-stat acid-base management to regulate carbon dioxide tension, hematocrit values of 25% to 30%, blood glucose levels less than 200 mg/dL, and the use of arterial line filters.

After induction of anesthesia and before skin incision, we performed remote ischemic preconditioning consisting of 3 cycles of 5-minute inflation of a blood pressure cuff to 200 mm Hg (or at least to a pressure 50 mm Hg higher than the systolic arterial pressure) to one upper arm, followed by 5-minute reperfusion with the cuff deflated. In patients assigned to the control group, sham remote ischemic preconditioning intervention was induced by 3 cycles of upper limb pseudo ischemia (low pressure, 5-minute blood pressure cuff inflation to a pressure of 20 mm Hg and 5-minute cuff deflation). The surgical procedure and perioperative care were performed according to the standard at each center.

Outcomes

Our primary end point was the occurrence of acute kidney injury within the first 72 hours after surgery. We defined acute kidney injury according to the Kidney Disease: Improving Global Outcomes criteria (eTable 2 in Supplement 2).[13] Secondary end points were severe acute kidney injury (stage 2-3) within 72 hours, 30-day all-cause mortality, need for renal replacement therapy during index hospitalization, duration of ventilator support, length of stay in the intensive care unit, length of hospital stay, in-hospital death, concentrations of various urinary biomarkers in the first 24 hours after surgery, and perioperative myocardial infarction and stroke during the index hospital stay.

We abstracted clinical variables from the medical record. Initiation of renal replacement therapy was at the discretion of the intensive care unit clinicians blinded to treatment assignment. Criteria for renal replacement therapy were not included in the protocol. Perioperative myocardial infarction and stroke were defined as described previously.[17] Perioperative myocardial infarction was defined as cardiac troponin I concentration in serum more than 5 times the 99th percentile of the reference range when associated with new left bundle-branch block pathologic Q waves, or angiography-confirmed new or native coronary occlusion. Postoperative myocardial infarction was defined as an increase in troponin complex I concentration from baseline to at least twice the upper limit of normal, together with evidence of myocardial ischemia, such as electrocardiographic changes or angina symptoms. Cerebrovascular accidents or stroke during or after hospital admission was assessed if at least 1 of the following criteria was fulfilled: a neurologic event resulting in new, temporary, or permanent focal or global neurologic deficit, any embolic event after the immediate perioperative period (when anesthesia-induced unconsciousness was completely reversed), or a stroke or permanent neurologic event lasting longer than 24 hours or less than 24 hours if a cerebral lesion was observed on imaging. Repeated revascularization was defined as any percutaneous coronary intervention or repeated coronary artery bypass graft surgery after the primary coronary artery bypass graft surgery.

Blood and Urine Sampling and Analysis

Blood samples were drawn before surgery and at prespecified points after surgery for measurement of serum creatinine concentrations (4 hours after cardiac surgery and on every morning for at least 3 days after cardiac surgery). We estimated glomerular filtration rate with the Modification of Diet in Renal Disease formula. Urine samples for biomarkers were collected before remote ischemic preconditioning or sham remote ischemic preconditioning, after inducing each one and at 4, 12, and 24 hours after surgery. Insulinlike growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases 2 (TIMP-2), both inducers of G1 cell cycle arrest, are implicated in acute kidney injury and serve as biomarkers to predict it. The product of urine TIMP-2 and IGFBP7 concentrations, (TIMP-2) × (IGFBP7), was measured with the NephroCheck Test (Astute Medical). Urine neutrophil gelatinase-associated lipocalin (NGAL), was measured with a commercially available assay (Dianova) according to the manufacturer's protocol. Urine high-mobility group box (HMGB) 1 was measured with a commercially available assay (http://antibodies-online.com) according to the manufacturer's protocol.

Statistical Analysis

We calculated a necessary sample size based on the primary end point, using nQuery Advisor version 7. The primary efficacy analysis was intended to show superiority of remote ischemic preconditioning in high-risk cardiac surgery patients, applying a 2-sided χ^2 test on significance level α=.05. According to an observational study we performed in a similar patient population,[18] the expected acute kidney injury rate in the control group treated with sham remote ischemic preconditioning was 50%. The expected absolute risk reduction for acute kidney injury was 18% according to a published single-center study investigating the effect of remote ischemic preconditioning on acute kidney injury after cardiac surgery.[10] As a result of these considerations and a power of 80%, the required sample size was calculated to be 117 evaluable patients per treatment group, ie, 234 in total. An additional 6 patients were recruited to account for loss to follow-up or nonevaluable data.

The primary efficacy analysis included all randomized patients (full analysis set) and was performed according to the intent-to-treat principle, ie, all patients were analyzed according to their randomization (see statistical analysis plan in Supplement 1). For the primary outcome and the secondary end points acute kidney injury severity, need for renal replacement therapy, and mortality, all patients had complete data. For the analysis of biomarkers over time, information of all patients who had evaluable data for the respective time was included. For the logistic regression analyses, only patients with complete data regarding the included covariates were included. No imputation of the data was performed. Descriptive statistics are summarized for categorical variables as frequency (%) and were compared between groups with χ^2 test (or Fisher exact test if the produced matrixes contained cells with expected counts <5). Continuous variables, expressed as mean (standard deviation), were compared between groups with an unpaired *t* test. Continuous variables, which were not distributed normally, were analyzed with nonparametric tests (Mann-Whitney *U* and Wilcoxon for unpaired and paired observations, respectively). We estimated the relative risk (RR) reduction and the absolute risk reduction, including 95% CIs,

for the occurrence of acute kidney injury, comparing the 2 study cohorts. The 95% CIs for median differences were calculated by bootstrapping (10 000 random samples taken equally distributed from both randomization groups).

To identify the association between various risk factors and acute kidney injury, we used multivariable logistic regression with acute kidney injury within 72 hours of surgery (yes or no) as the dependent variable. We included variables from the Cleveland Clinic Foundation score[14] (age, sex, diabetes, chronic obstructive pulmonary disease, previous heart surgery, and preoperative creatinine level), along with HMGB-1 (TIMP-2) × (IGFBP7) (difference between pre- and post-remote ischemic preconditioning) and remote ischemic preconditioning as dependent variables, using backward likelihood ratios for variable retention in the model. We used the Wald test and reported *P* value odds ratios with 95% CIs. To identify factors associated with (TIMP-2) × (IGFBP7) immediately after remote ischemic preconditioning, we used a predefined cutoff of 0.5 ng/mL^2/1000[18] and used multivariable logistic regression with the same variables as described above (except [TIMP-2] × [IGFBP7]) as independent variables. Model performance was assessed by the analysis of the area under the receiver operating characteristic curve. *P* value is given for the hypothesis test area under the curve = 0.5. IBM SPSS version 21.0 software was used. Two-sided *P* values ≤.05 were considered indicative of statistical significance.

Results

Patients

Of 790 patients screened for the trial, 240 were enrolled and randomized to receive either remote ischemic preconditioning (n = 120) or sham remote ischemic preconditioning (control) (n = 120) and included in the primary analysis (Figure 1). The baseline and intraoperative characteristics were similar between the groups (**Table 1**). The number of patients with a low ejection fraction was similar between the groups.

Primary Outcome

Significantly fewer patients in the remote ischemic preconditioning arm developed acute kidney injury within 72 hours after surgery compared with the control group (37.5% vs 52.5%; *P* = .02; RR, 71%; 95% CI, 54%-95%; absolute risk reduction, 15.0%; 95% CI, 2.56%-27.44%; RR reduction, 28.6%; 95% CI, 5%-47%) (**Table 2**). Correction of serum creatinine level for fluid balance slightly changed the occurrence of acute kidney injury but did not change the difference in acute kidney injury rate between the remote ischemic preconditioning group and control group (42.5% vs 53.3%; *P* = .03). We performed a stratified analysis of the primary end point to check for site effects, using the Cochran and Mantel-Haenszel *t* test. The 2-sided *P* value of the test was .02 (OR, 0.56; 95% CI, 0.33-0.93).

Secondary Outcomes

Remote ischemic preconditioning significantly reduced the number of moderate and severe acute kidney injury cases compared with that of the control group (12.5% vs 25.8%; *P* = .02; RR, 85%; 95% CI, 75%-97%) but did not reduce the rate of mild acute kidney injury (25% vs 26.7%; *P* = .77; RR, 98%; 95% CI, 84%-114%). Use of renal replacement therapy (5.8% vs 15.8%; *P* = .01; absolute risk reduction, 10%; 95% CI, 2.25%-17.75%) and length of intensive care unit stay (3 days [interquartile range, 2-5] vs 4 days [interquartile range, 2-7]; 95% CI, 0-2 days, median difference; *P* = .04) were significantly reduced with remote ischemic preconditioning (Table 2). However, we found no significant differences between groups in time receiving mechanical ventilation, myocardial infarction, and perioperative stroke (Table 2). Length of hospital stay after surgery was comparable. All-cause in-hospital mortality and 30-day mortality were not different between groups (Table 2).

Biomarkers

Although baseline urinary (TIMP-2) × (IGFBP7) and NGAL, tested immediately before the intervention, did not differ between groups, the control group had significantly higher urinary (TIMP-2) × (IGFBP7) at 4 hours after (remote ischemic preconditioning, 0.36 vs control, 0.97 ng/mL^2/1000; difference, 0.61 [95% CI, 0.27-0.86]; *P*<.001) and 12 hours after cardiopulmonary bypass (*P* < .001) and higher NGAL at 4 hours after cardiopulmonary bypass (*P* = .04) compared with the remote ischemic preconditioning group (**Figure 2**A and B).

By contrast, remote ischemic preconditioning increased urinary (TIMP-2) × (IGFBP7) immediately after remote ischemic preconditioning before cardiopulmonary bypass compared with that of the control group (Figure 2A), whereas urinary NGAL was unchanged (Figure 2B). Patients with urinary (TIMP-2) × (IGFBP7) level greater than or equal to 0.5 ng/mL^2/1000 before the initiation of the cardiopulmonary bypass had a significantly reduced rate of acute kidney injury compared with patients with lower urinary (TIMP-2) × (IGFBP7) concentration (RR, 67%; 95% CI, 53%-83%; *P* < .001) (eFigure 1A in Supplement 2). However, patients with urinary (TIMP-2) × (IGFBP7) greater than or equal to 0.5 ng/mL^2/1000 4 hours after cardiopulmonary bypass had a significantly increased rate of acute kidney injury compared with patients with lower urinary (TIMP-2) × (IGFBP7) (RR, 299%; 95% CI, 188%-473%; *P* < .001) (eFigure 1B in Supplement 2).

High-mobility group box 1, a damage-associated molecular pattern, was measured at baseline and after the intervention before cardiopulmonary bypass. Urinary HMGB-1 was similar in both groups at baseline. However, it significantly increased immediately after remote ischemic preconditioning (Figure 2C). In multivariable logistic regression analysis, preoperative serum creatinine level and previous heart surgery were associated with increased risk for acute kidney injury, whereas post–remote ischemic preconditioning HMGB-1 (OR, 0.75; 95% CI, 0.61-0.91; *P* = .005) and (TIMP-2) × (IGFBP7) (OR, 0.57; 95% CI, 0.35-0.94; *P* = .03) were associated with lower risk for acute kidney injury (**Table 3**). Furthermore, both HMGB-1 and remote ischemic preconditioning were significant predictors of post–remote ischemic preconditioning (TIMP-2) × (IGFBP7) ≥ 0.5 ng/mL^2/1000 (Table 3).

Table 1. Baseline and Operative Characteristics

	Control (n = 120)	RIPC (n = 120)
Age, mean (SD), y	70.6 (9.9)	70.1 (9.1)
Male sex, No. (%)	75 (62.5)	76 (63.3)
ASA grade, No. (%)[a]		
1	0	0
2	24 (20.0)	27 (22.5)
3	88 (73.3)	86 (71.7)
4	8 (6.7)	7 (5.8)
New York Heart Association class, No. (%)		
I	6 (5.4)	5 (4.5)
II	28 (25.0)	32 (28.8)
III	60 (53.6)	57 (51.4)
IV	18 (16.1)	17 (15.3)
Cleveland Clinic Foundation score, median (IQR), points[b]	6 (6-6)	6 (6-6)
Preoperative creatinine, mean (SD), mg/dL	1.2 (0.4)	1.1 (0.4)
eGFR, mean (SD), mL/min/1.73 m^2	56.4 (15.8)	56.7 (13.4)
Comorbidities, No. (%)		
Hypertension	116 (96.7)	116 (96.7)
Congestive heart failure	101 (84.2)	101 (84.2)
Diabetes	44 (36.7)	46 (38.3)
Chronic obstructive pulmonary disease	40 (33.3)	36 (30.0)
Chronic kidney disease	39 (32.5)	35 (29.2)
Previous heart surgery	14 (11.7)	13 (10.8)
Left ventricular ejection fraction <35%	13 (10.8)	23 (19.2)
Medication, No. (%)		
Aspirin	66 (55.0)	77 (64.2)
Clopidogrel	15 (12.5)	11 (9.2)
β-Blockers	78 (65.0)	68 (56.7)
Statins	85 (70.8)	80 (66.7)
Diuretics	71 (59.2)	63 (52.5)
ACE inhibitors or ARBs	73 (60.8)	71 (59.2)
Intraoperative times, median (IQR), min		
Aortic cross-clamp	78.0 (58.5-112.0)	86.0 (65.0-105.0)
Cardiopulmonary bypass	116.0 (89.5-165.0)	120.0 (99.5-150.0)
Procedure, No. (%)		
CABG only	36 (30.0)	44 (36.7)
Valve only	21 (17.5)	28 (23.3)
Combined or other	63 (52.5)	48 (40.0)
Baseline urine biomarkers, median (IQR)		
Urine (TIMP-2) × (IGFBP7), ng/mL^2/1000	0.2 (0.1-0.5)	0.3 (0.1-0.7)
Urine NGAL, ng/mL	10.7 (4.5-30.5)	9.9 (4.9-25.2)
Urine HMGB-1, ng/mL	0 (0-0)	0 (0.0-20.5)
(TIMP-2) × (IGFBP7) ≥0.5, No. (%)	31 (26.3)	40 (33.6)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ASA, American Society of Anesthesiology; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; HMGB, high-mobility group box; IGFBP7, insulinlike growth factor–binding protein 7; NGAL, neutrophil gelatinase-associated lipocalin; RIPC, remote ischemic preconditioning; TIMP, tissue inhibitor of metalloproteinases.

[a] American Society of Anesthesiology grades: 1, healthy patient; 2, mild systemic disease that does not limit physical activity; 3, severe systemic disease that limits physical activity; and 4, severe systemic disease that is a constant threat to life (grade 5 patients were not eligible for inclusion).

[b] The Cleveland Clinic Foundation score (0-17 points) is composed of 13 preoperative risk factors, including patient characteristics, comorbidities, and type of surgery. A higher number correlates with a higher rate of dialysis-dependent acute kidney injury after cardiac surgery.

Discussion

The results of this multicenter, randomized, double-blind, clinical trial confirm the findings of a previous single-center study that remote ischemic preconditioning reduces the rate of acute kidney injury after cardiac surgery in high-risk patients.[10] In our study, the intervention achieved more than a 15% absolute reduction in the rate of perioperative acute kidney injury. Especially the occurrence of moderate and severe acute kidney injury was reduced by remote ischemic preconditioning. We furthermore showed a benefit from remote ischemic preconditioning with a reduced use of renal replacement therapy and a shorter length of intensive care unit stay. Finally, we found that remote ischemic preconditioning reduced the post-cardiopulmonary bypass expression of biomarkers of acute kidney injury, including neutrophil gelatinase-associated lipocalin and the recently approved biomarker panel (TIMP-2) × (IGFBP7). Remote ischemic preconditioning increased pre-cardiopulmonary bypass release of the "alarm"

Table 2. Primary and Secondary Study Outcomes, by Group

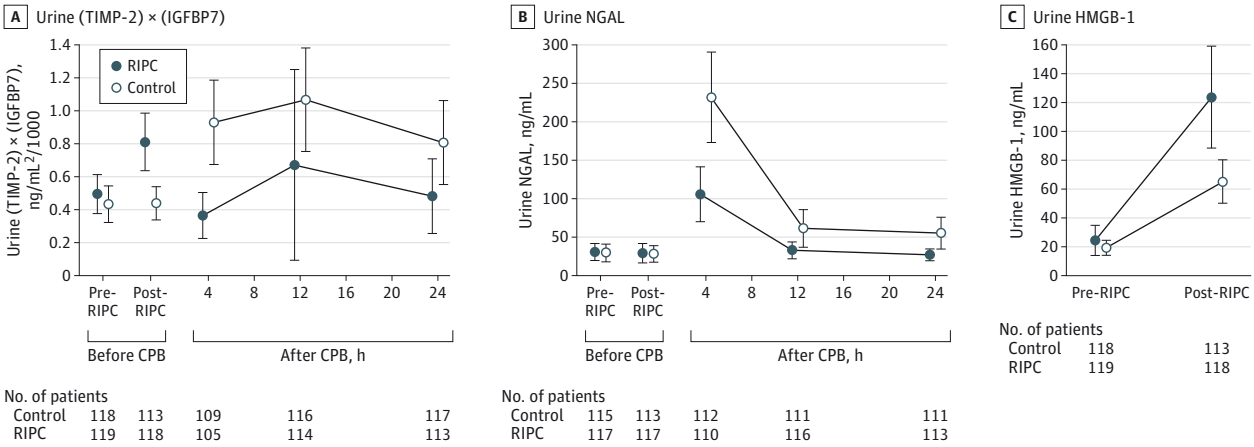
	Control (n = 120)	RIPC (n = 120)	ARR or Median Difference (95% CI)	*P* Value
Primary Outcome, No. (%)				
AKI within 72 h	63 (52.5)	45 (37.5)	15 (2.56 to 27.44)	.02
AKI stage				
1	32 (26.7)	30 (25)		
2	14 (11.7)	8 (6.7)		
3	17 (14.2)	7 (5.8)		
Secondary Outcomes				
RRT, No. (%)	19 (15.8)	7 (5.8)	10 (2.25 to 17.75)	.01
Mechanical ventilation, median (IQR), h	15 (12-21)	14 (11-21)	1 (−1.54 to 4)[a]	.16
Intensive care unit stay, median (IQR), d	4 (2-7)	3 (2-5)	1 (0 to 2)[a]	.04
Hospital stay, median (IQR), d	13 (10-19)	12 (9-19)	1 (−2 to 2.5)[a]	.45
In-hospital death, No. (%)	4 (3.3)	6 (5.0)	1.67 (0 to 6.72)[b]	.54
30-d mortality, No. (%)	5 (4.2)	7 (5.8)	1.67 (0 to 7.18)[b]	.77
Myocardial infarction, No. (%)	5 (4.2)	6 (5.0)	0.83 (0 to 6.12)[b]	.76
Stroke, No. (%)	3 (2.5)	2 (1.7)	0.83 (0 to 4.45)[b]	.65

Abbreviations: AKI, acute kidney injury; ARR, absolute risk reduction; RIPC, remote ischemic preconditioning; RRT, renal replacement therapy.

[a] Bootstrapped 95% CI.

[b] Estimation of lower limit <0.

Figure 2. Analysis of Acute Kidney Injury Biomarkers



A, Analysis of urine (TIMP-2) × (IGFBP7) before and after remote ischemic preconditioning (RIPC) and cardiopulmonary bypass (CPB) (pre-RIPC, *P* = .33; post-RIPC, *P* = <.01; 4 h after CPB, *P* = .01; 12 h after CPB, *P* = .01; 24 h after CPB, *P* = .35) (lower and upper limit of the reference range, 0.03 [2.5 percentile] and 1.93 [97.5 percentile], respectively). B, Analysis of urine neutrophil gelatinase-associated lipocalin (NGAL) concentrations before and after RIPC and CPB (pre-RIPC, *P* = .79; post-RIPC, *P* = .72; 4 h after CPB, *P* = .04; 12 h after CPB, *P* = .74; 24 h after CPB, *P* = .28) (reference range, 153 ng/mL; 90% CI, 142 to 182 ng/mL). C, Analysis of HMGB-1 concentrations before and after RIPC (pre-RIPC, *P* = .23; post-RIPC, *P* = <.01) (reference range: mean, 0.39 ng/mL; upper limit of the reference range, 1.4 ng/mL [97.5 percentile]). Error bars indicate 95% CI. All *P* values are for comparison of RIPC vs control. HMGB-1 indicates high-mobility group box 1; (TIMP-2) × (IGFBP7) indicates the product of urine IGFBP7 (insulinlike growth factor–binding protein 7) and TIMP-2 (tissue inhibitor of metalloproteinases 2).

markers (TIMP-2) × (IGFBP7) while having no effect on the damage marker neutrophil gelatinase-associated lipocalin. This scenario is consistent with the known roles of TIMP-2 and IGFBP7 in the induction of G1 cell-cycle arrest, an epithelial defense mechanism.[19]

Several small feasibility and controlled clinical trials provided evidence that remote ischemic preconditioning can reduce myocardial injury during coronary bypass surgery,[20] during surgical repair of congenital heart defects,[21] and before percutaneous coronary interventions.[22] Two studies have reported a protective effect of remote ischemic preconditioning on renal function.[10,23] In contrast to these studies, 3 other trials failed to demonstrate renal protection with remote ischemic preconditioning (see eFigure 2 in the Supplement).[9,11,24] Our study provides new insight into the heterogeneity of treatment effect observed across these trials. Although the mechanisms responsible for the benefit of remote ischemic preconditioning are not completely understood, one possible explanation is that damage-associated molecular patterns released from the ischemic tissue engage self-protective mechanisms in the kidney such as cell-cycle arrest (see eFigure 3 in the Supplement). We measured HMGB-1, a well-known damage-associated molecular pattern, in urine and found that remote ischemic preconditioning resulted in increased release of this molecule. Early increases in HMGB-1 and (TIMP-2) × (IGFBP7) were strongly associated with lower risk

Table 3. Predictors of Acute Kidney Injury (AKI) and Concentration of Tissue Inhibitor of Metalloproteinases × Insulinlike Growth Factor–Binding Protein 7 Increase, Using Multivariable Logistic Regression

Variable[a]	No. of AKI Events/ No. (%) of Patients	Odds Ratio (95% CI)	*P* Value
AKI (n = 239)			
Previous heart surgery			
Yes	17/27 (63.0)	2.25 (0.94-5.40)	.07
No [reference]	91/213 (42.7)		
Preoperative creatinine, mg/dL		2.29 (1.11-4.71)	.03
HMGB-1 after RIPC, ng/mL/100		0.75 (0.61-0.91)	.005
(TIMP-2) × (IGFBP7) difference, $(ng/mL)^2/1000$[b]		0.57 (0.35-0.94)	.03
(TIMP-2) × (IGFBP7) ≥0.5[c] (n = 229)			
HMGB-1 after RIPC, ng/mL/100		1.20 (1.02-1.41)	.03
RIPC			
Yes [reference]	45/120 (37.5)	3.70 (2.07-6.62)	.001
No	36/120 (52.5)		

Abbreviations: HMGB-1, high-mobility group box 1; IGFBP7, insulinlike growth factor–binding protein 7; RIPC, remote ischemic preconditioning; TIMP-2, tissue inhibitor of metalloproteinases 2.

[a] For continuous variables, the reference increment is 1 per given unit.

[b] The (TIMP2) × (IGFBP7) value immediately after the intervention (before initiation of cardiopulmonary bypass) minus the (TIMP-2) × (IGFBP7) value before the intervention. C statistic (area under the receiver operating characteristic [AUC]), 0.718; 95% CI, 0.65-0.78; *P* = .001.

[c] C statistic (AUC), 0.62; 95% CI, 0.55-0.69; *P*=.001.

for acute kidney injury. However, not all patients responded to remote ischemic preconditioning with increased HMGB-1 release or increases in urine (TIMP-2) × (IGFBP7). Future studies of this intervention might benefit from monitoring these biomarkers.

Differences in outcomes across remote ischemic preconditioning trials might also have been due to differences in study protocols, confounding comorbidities, anesthetic regimens, and surgical technique. Because ours is the first study to our knowledge to measure biomarkers, it is not possible to know whether previous trials with negative results failed to induce changes in these intermediate end points. In our protocol, patients with diabetes treated with sulfonylurea medications were excluded because these drugs inhibit adenosine triphosphate-sensitive potassium-channel conductance and may impede the effects of remote ischemic preconditioning.[25] Although volatile anesthetics might have a preconditioning effect,[26,27] we excluded the use of propofol because it may mitigate the effects of remote ischemic preconditioning.[15] In our trial, we included only patients with a high risk for acute kidney injury, as identified by a Cleveland Clinic Foundation score greater than or equal to 6.[14] We focused on this particularly high-risk patient population because 2 consensus conferences concluded that they would be most likely to benefit from remote ischemic preconditioning.[28]

The pathophysiology of acute kidney injury is complex and still incompletely understood. New evidence suggests that adaptive responses by tubular epithelial cells to injurious signals are responsible for renal dysfunction and that renal inflammation and microcirculatory dysfunction further amplify these mechanisms.[7,29] Remote ischemic preconditioning induces the release of various molecules that appear to mediate the protective effect of this intervention.[5] Here, we demonstrate that these mediators might be inducing G1 cell-cycle arrest in the kidney, as indicated by increased urinary (TIMP-2) × (IGFBP7) after remote ischemic preconditioning. Cell-cycle arrest has been implicated in acute kidney injury,[30,31] and urinary (TIMP-2) × (IGFBP7) has been shown to be predictive of acute kidney injury in patients undergoing cardiac surgery,[18] as well as in general intensive care unit populations.[32] However, cell-cycle arrest is a self-defense mechanism. When exposed to stress, epithelial cells may enter a short period of G1 cell-cycle arrest[19] until the danger has passed or injury has been repaired. High-mobility group box-1 is an endogenous damage-associated molecular pattern molecule that can serve as an early mediator in the context of sterile inflammation, with release occurring as a consequence of acute cellular stress, hypoxia, or necrosis.[33] Extracellular HMGB-1 can bind to several pattern recognition receptors, including Toll-like receptors, which can directly or indirectly induce cell-cycle arrest.[29] Our data are in line with those of a recent animal study demonstrating that preconditioning with recombinant HMGB-1 provides protection against acute kidney injury.[34] We hypothesized that HMGB-1 (and other damage-associated molecular patterns) is released after remote ischemic preconditioning and these molecules induce cell-cycle arrest in tubular epithelial cells (see eFigure 3). Increases in urine (TIMP-2) × (IGFBP7) immediately after remote ischemic preconditioning should therefore be protective from subsequent kidney injury induced by cardiac surgery, whereas late increases in these markers (for example, after cardiopulmonary bypass) should herald acute kidney injury. Our results fit this scenario exactly.

In cardiac surgery, perioperative acute kidney injury is closely associated with postoperative morbidity and mortality in the short and long term.[2,35-37] Several studies demonstrated an association between acute kidney injury and increased morbidity, short-term and long-term mortality, and use of resources in various patient populations.[2,35-41] This relationship holds true even with small increases of serum creatinine level for cardiac surgery patients.[2,35,39,41] Remote ischemic preconditioning could thus represent a simple and promising strategy to provide protection to the kidney and improve postoperative outcomes. Such measures would be particularly desirable to deal with the increasingly challenging risk profiles of patients who are referred for cardiac surgery.

Study Limitations

Our study is not without limitations. Although this was a multicenter trial, it was adequately powered only to analyze prospectively the rate of perioperative acute kidney injury and thus

a phase 2 equivalent study. The secondary end points, for which the study was not powered but which were assessed in view of a significant effect on the primary end point, indicated reduced kidney damage (by acute kidney injury stage, as well as by urinary (TIMP-2) × (IGFBP7) and [neutrophil gelatinase-associated lipocalin]) in patients undergoing remote ischemic preconditioning. The use of renal replacement therapy was reduced in the intervention group as well. Although the critical care physicians treating the patients were blinded to the study group allocation, initiation of renal replacement therapy was at their discretion. Among critically ill patients with acute kidney injury, the timing of renal replacement therapy initiation remains an area of considerable controversy.[13] Another limitation of this study is that although we have found important associations with intermediary end points, we cannot prove mechanism. Future experimental and clinical studies are needed to better establish the relationship between damage-associated molecular patterns, cell-cycle arrest, and rate or severity of acute kidney injury. Likewise, future studies will need to address the optimal methods for remote ischemic preconditioning and whether benefits are consistent across patients with various risks for acute kidney injury, such as those with preexisting chronic kidney disease or with lower Cleveland Clinic Foundation score. We did not detect a reduction in mortality between the 2 groups; as expected, this secondary end point is uncommon and our study was too small. According to our 30-day mortality results, we would need more than 4000 patients (183 deaths) to detect a difference in the mortality with 80% power. It remains to be determined whether preventing cardiac surgery–associated acute kidney injury with remote ischemic preconditioning will reduce morbidity, mortality, and use of resources other than renal replacement therapy.

Conclusions

Among high-risk patients undergoing cardiac surgery, remote ischemic preconditioning compared with control significantly reduced the rate of acute kidney injury and use of renal replacement therapy. The observed reduction in the rate of acute kidney injury and the need for renal replacement warrant further investigation.

ARTICLE INFORMATION

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