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Ischemic preconditioning at a remote site prevents acute kidney injury in patients following cardiac surgery

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Acute kidney injury, a common complication of cardiac surgery with cardiopulmonary bypass, is associated with increased morbidity and mortality. Ischemic preconditioning at a remote site mitigates ischemia–reperfusion injury and may prevent acute kidney injury after cardiac surgery, thus providing clinical benefit. To further study this, we enrolled 120 adult patients undergoing elective cardiac surgery for whom cardiopulmonary bypass was anticipated in a randomized, single-blind, and controlled pilot trial. Patients were stratified for the type of surgery and equally assigned to a control group or to receive remote ischemic preconditioning by an automated thigh tourniquet consisting of three 5-min intervals of lower extremity ischemia separated by 5-min intervals of reperfusion. The primary end point was acute kidney injury defined as an elevation of serum creatinine of $\geqslant$0.3 mg/dl or $\geqslant$50% within 48 h after surgery. Fifty-nine patients in each group were analyzed on an intention-to-treat basis. Acute kidney injury occurred in 12 remote ischemic preconditioned and 28 control patients, reflecting an absolute risk reduction of 0.27 and a significantly reduced relative risk due to preconditioning of 0.43. Hence, remote ischemic preconditioning prevents acute kidney injury in patients undergoing cardiopulmonary bypass-assisted cardiac surgery.

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Acute kidney injury, a significant complication of cardiac surgery, is occurring with greater frequency as patients at high risk for complications are increasingly referred for surgery.[1,2] Depending on how it is defined, acute kidney injury occurs in up to 45% of patients after cardiac surgery, and approximately 1 to 2% require renal replacement therapy.[3–5] Patients with acute kidney injury after cardiac surgery are at risk for lengthened intensive care unit and hospital stays and for short-term and long-term mortality.[3,5–11] Recognizing that even small increases in serum creatinine levels are associated with increased morbidity and mortality, the Acute Kidney Injury Network (AKIN) has recommended an acute increase in the serum creatinine concentration of $\geqslant$0.3 mg/dl as a diagnostic criterion for acute kidney injury.[12]

Cardiopulmonary bypass is employed in most cardiac surgeries, and although the mechanisms are not fully understood, ensuing ischemic, inflammatory, and oxidative injuries to renal tubular epithelial cells have been implicated in the cause of acute kidney injury.[13] Despite numerous clinical trials of pharmacologic interventions, a means to prevent acute kidney injury associated with cardiac surgery has remained elusive.[13–16]

Remote ischemic preconditioning is a phenomenon in which ischemia–reperfusion injury of an organ is mitigated by previous application of brief ischemic intervals in a distant organ or limb.[17,18] Several encouraging trials of remote ischemic preconditioning have suggested clinical benefit.[19–25] Because the mechanisms of ischemia–reperfusion injury[26,27] are similar to those proposed for acute kidney injury after cardiopulmonary bypass, we tested the hypothesis that remote ischemic preconditioning prevents acute kidney injury in patients undergoing cardiac surgery.

RESULTS

Study patients

We randomized 120 patients to remote ischemic preconditioning ($n = 60$) and control ($n = 60$) groups: 79 scheduled for coronary artery bypass grafting (CABG), 18 for valve

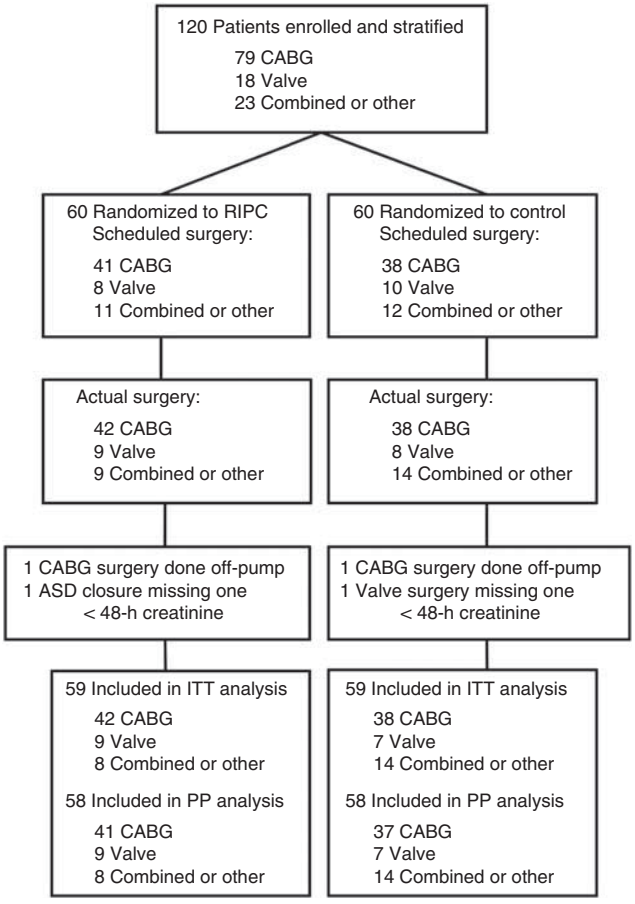

Figure 1 | Patient flowchart. Enrolled patients are stratified by scheduled surgery and randomized to study groups. The coronary artery bypass grafting (CABG) patients operated off-pump were included in the intention-to-treat (ITT) analysis. ASD, atrial septal defect; PP, per-protocol; RIPC, remote ischemic preconditioning.

surgery, and 23 for combined CABG and valve or other surgery (Figure 1). When scheduled and actual surgeries were different, actual surgeries were used for the intention-to-treat and per-protocol analyses. One CABG patient was randomized from a block used out of sequence. After exclusion of 2 patients (1 remote ischemic preconditioning patient and 1 control patient for whom second postoperative day serum creatinine levels were not obtained), 59 patients in each group were included in the intention-to-treat analysis. Two patients who underwent CABG without cardiopulmonary bypass were excluded in the per-protocol analysis.

The remote ischemic preconditioning and control groups were not significantly different with respect to baseline characteristics (Table 1) and operative data (Table 2), except that more patients in the control group had a history of previous cardiac surgery. Prediction scores for acute kidney injury requiring renal replacement therapy were similar between groups, as were the proportions of patients at moderate or high risk (score >2).

Primary outcome

Significantly fewer patients in the remote ischemic preconditioning group had acute kidney injury within 48 h after surgery compared with the control group (20% vs 47%; $P=0.004$; relative risk, 0.43; 95% confidence interval (CI), 0.24–0.76; absolute risk reduction, 0.27; 95% CI, 0.10–0.42) (Table 3). Per-protocol analysis confirmed this result (21% vs 48%; $P=0.003$; relative risk, 0.43; 95% CI, 0.24–0.76).

Because significantly more patients in the control group than in the intervention group had a history of previous cardiac surgery, we performed an additional χ^2 analysis stratified on previous cardiac surgery history, with nearly identical results (relative risk, 0.45; $P=0.004$).

Secondary outcomes

Median change in plasma neutrophil gelatinase-associated lipocalin (NGAL) levels, drawn immediately before the preconditioning regimen and 3 h after cardiopulmonary bypass, did not differ significantly between the remote ischemic preconditioning and control groups (39 vs 43 ng/ml, respectively; $P=0.46$). We observed a nonsignificant difference in the incidence of postoperative oliguria during the initial 12 h after surgery between remote ischemic preconditioning and control groups (7% vs 2%, respectively; $P=0.36$; Table 3). Per-protocol analysis remained nonsignificant for both outcomes (data not shown).

Other renal outcomes

In a *post hoc* analysis of patients who had acute kidney injury, 11 (19%) and 23 (39%) patients had stage I acute kidney injury, and 1 (2%) and 5 (8%) patients had stage II acute kidney injury in the remote ischemic preconditioning and control groups, respectively ($P=0.005$; Table 3). No patients had stage III acute kidney injury within the first postoperative 48 h or required renal replacement therapy before hospital discharge.

Significantly fewer patients in the remote ischemic preconditioning group had sustained acute kidney injury for at least 2 consecutive days compared with the control group (17% vs 36%; $P=0.04$). Sustained acute kidney injury for at least 3 consecutive days occurred less frequently in the remote ischemic preconditioning group (8% vs 19%), but this difference was not statistically significant ($P=0.18$; Table 3).

Other postoperative outcomes and adverse events

We found no significant differences between groups in the use of vasoactive drugs, prolonged mechanical ventilation, or need for reintubation. No patients had postoperative myocardial infarction. One patient in the remote ischemic preconditioning group had postoperative congestive heart failure. Length of intensive care unit stay and hospital stay after surgery were comparable. One patient in the control group died 2 days after surgery (Table 3).

Table 1 | Baseline characteristics of the study patients according to intervention group

	RIPC (*n*=59)	Control (*n*=59)	*P*-value
Age, year	62 ± 9[a]	65 ± 11	0.14
Male sex, no. (%)	41 (69)	40 (68)	0.84
Preoperative creatinine, mg/dl[b]	0.93 ± 0.24	0.95 ± 0.31	0.68
GFR <60 ml/min per 1.73 m^2, no. (%)[c]	10 (17)	9 (15)	0.80
Comorbidities			
Hypertension, no. (%)	44 (75)	50 (85)	0.25
Diabetes mellitus	24 (41)	21 (36)	0.70
Insulin requiring, no. (%)	9 (15)	5 (8)	0.39
Congestive heart failure, no. (%)	10 (17)	9 (15)	0.80
Peripheral vascular disease, no. (%)	8 (14)	10 (17)	0.80
Chronic obstructive pulmonary disease, no. (%)	11 (19)	5 (8)	0.18
Previous heart surgery, no. (%)	6 (10)	15 (25)	0.05
Left ventricular ejection fraction <35%, no. (%)	8 (14)	4 (7)	0.36
Coronary angiogram ⩽2 days before, no. (%)	18 (31)	15 (25)	0.68
Preoperative intraaortic balloon pump, no. (%)	3 (5)	1 (2)	0.62[d]
Preoperative medications			
Sulfonylurea, no. (%)	6 (10)	3 (5)	0.49[d]
ACE-I or ARB, no. (%)	14 (24)	14 (24)	—
NSAID or COX-2 inhibitor, no. (%)	2 (3)	0 (0)	0.50[d]
Acute kidney injury propensity score			
Median (range)	2 (0, 5)	2 (0, 9)	0.77[e]
Score denoting moderate or high risk, no. (%)	16 (27)	20 (34)	0.55

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; COX-2, cyclooxygenase 2; GFR, glomerular filtration rate; NSAID, nonsteroidal anti-inflammatory drug; RIPC, remote ischemic preconditioning.
[a]The ± values are means ± s.d.
[b]To convert values for creatinine to μmol/l, multiply by 88.4.
[c]GFR is calculated by a simplified Modification of Diet in Renal Disease (MDRD) formula.
[d]Fisher's exact test.
[e]Mann–Whitney *U* test.

Table 2 | Operative characteristics of the patients according to intervention group

	RIPC (*n*=59)	Control (*n*=59)	*P*-value
CPB time, min	115 ± 37[a]	113 ± 37	0.84
Aortic cross-clamp time, min	73 ± 38	68 ± 35	0.54
Isoflurane anesthesia, no. (%)	59 (100)	59 (100)	—
Type of surgery			
CABG only, no. (%)	42 (71)	38 (64)	0.35
Valve only, no. (%)	9 (15)	7 (12)	
Combined or other, no. (%)	8 (14)	14 (24)	

Abbreviations: CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; RIPC, remote ischemic preconditioning.
[a]The ± values are means ± s.d.

DISCUSSION

Our study shows that remote ischemic preconditioning prevents acute kidney injury in patients undergoing cardiac surgery with cardiopulmonary bypass. We observed a 27% absolute risk reduction in the primary end point of an acute increase in serum creatinine levels by at least 0.3 mg/dl or 50% more than the baseline value within 48 h of surgery. Sustained acute kidney injury in which serum creatinine levels remained elevated for at least 2 consecutive days was also significantly reduced in the remote ischemic preconditioning group.

Several studies have described an association between acute kidney injury and increased morbidity, short-term and long-term mortality, and use of resources in various patient populations.[3,5–11,28–30] These relationships hold true even with small increases of serum creatinine levels for hospital inpatients and patients who have undergone cardiac surgery.[5,7–9,28,30] Moreover, the associations persist after adjustment for comorbidities and other factors predisposing to acute kidney injury, suggesting that even mild and clinically transient acute kidney injury has lasting effects on patient survival rather than simply being a marker for comorbidities and other contributors to mortality. Establishment of a method to prevent acute kidney injury in at-risk patients could substantially improve health outcomes.

Remote ischemic preconditioning has shown promise in randomized, controlled trials as a means for myocardial protection during coronary bypass surgery,[20,23] surgical repair of congenital heart defects,[19] and before percutaneous coronary interventions.[22,24] Two studies have reported a protective effect of remote ischemic preconditioning on renal function. In a randomized, controlled trial, Ali *et al.*[21] observed a relative risk for postoperative 'renal impairment' (that is, serum creatinine level >2.0 mg/dl) of 0.25 in patients who had undergone abdominal aortic aneurysm repair and received preconditioning before surgery. Venugopal *et al.*[25] retrospectively analyzed two randomized controlled

Table 3 | Postoperative outcomes according to intervention group

	RIPC (n=59)	Control (n=59)	P-value
Primary outcome			
AKI, no. (%)	12 (20)	28 (47)	0.004
Secondary outcomes[a]			
Change in plasma NGAL, ng/ml[b]	39 (−58, 284)	43 (−47, 203)	0.46[c]
Oliguria, no. (%)	4 (7)	1 (2)	0.36[d]
Other renal outcomes			
Sustained AKI			
⩾2 days, no. (%)	10 (17)	21 (36)	0.04
⩾3 days, no. (%)	5 (8)	11 (19)	0.18
AKI by AKIN stage			
No AKI, no. (%)	47 (80)	31 (53)	0.005[d]
Stage I, no. (%)	11 (19)	23 (39)	
Stage II, no. (%)	1 (2)	5 (8)	
Stage III, no. (%)	0 (0)	0 (0)	
RRT, no. (%)	0 (0)	0 (0)	—
Interventions			
Vasoactive drugs, 0–48 h, no. (%)	48 (81)	43 (73)	0.38
Vasoactive drugs at 48 h, no. (%)	22 (37)	20 (34)	0.85
MV >48 h, no. (%)	3 (5)	4 (7)	>0.99[d]
Reintubation, no. (%)	2 (3)	2 (3)	—
Complications			
Myocardial infarction	0 (0)	0 (0)	—
Congestive heart failure	1 (2)	0 (0)	>0.99[d]
Hospital outcomes			
ICU stay >3 days, no. (%)	12 (20)	8 (14)	0.46
Hospital stay after surgery, days[b]	7 (3, 35)	6 (4, 49)	0.39[c]
Hospital death, no. (%)	0 (0)	1 (2)	>0.99[d]

Abbreviations: AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; ICU, intensive care unit; MV, mechanical ventilation; NGAL, neutrophil gelatinase-associated lipocalin, RIFLE, risk, injury, failure, loss, and end-stage disease; RIPC, remote ischemic preconditioning; RRT, renal replacement therapy.
[a]NGAL data were analyzed for 53 RIPC patients and 55 control patients. Oliguria data were analyzed for 56 RIPC patients and 57 control patients.
[b]Reported as median (range).
[c]Mann–Whitney U test.
[d]Fisher's exact test.

trials of remote ischemic preconditioning for myocardial protection during coronary artery bypass graft surgery. The relative risk for acute kidney injury, defined by AKIN criteria, in patients who received the preconditioning regimen was 0.42, a finding of marginal statistical significance ($P = 0.10$). In contrast to our findings and those of Ali *et al.*[21] and Venugopal *et al.*,[25] a randomized controlled trial of remote ischemic preconditioning in 162 patients undergoing coronary artery bypass graft surgery while on cardiopulmonary bypass failed to demonstrate renal protection with preconditioning.[31] Postoperative serum creatinine was not measured until the fourth day following surgery. A protective effect of remote ischemic preconditioning on renal function may therefore have gone undetected if a significant number of subjects had acute kidney injury that began to resolve within the first 4 days after surgery.

How remote ischemic preconditioning protects against ischemia–reperfusion injury is uncertain. Evidence exists for neural and humoral mediators of signal transduction between the preconditioning stimulus and target organ. The most downstream events appear to be the same as for local preconditioning: preservation of mitochondrial function by opening of mitochondrial or plasmalemmal adenosine-5′-triphosphate (ATP)-sensitive potassium channels and closure of the mitochondrial permeability transition pore.[32] Sulfonylurea medications, which inhibit ATP-sensitive potassium channel conductance, may impede the effects of remote ischemic preconditioning.[33] We chose not to exclude from our study diabetic patients taking sulfonylureas to keep the study relevant to current clinical practice. Of the study patients, <10% took sulfonylureas within 48 h of their surgery. Although isoflurane might have had a preconditioning influence,[34,35] we chose not to modify the anesthetic regimen to maintain the clinical relevance of the study.

Although an increase in serum creatinine levels remains the standard by which acute kidney injury is defined, the sensitivity of serum creatinine values to so-called prerenal states is a significant shortcoming, particularly after cardiac surgery, when patients often exhibit significant cardiovascular instability. NGAL, which is heavily expressed in the kidney shortly after ischemic injury, appears not to be sensitive to hemodynamic changes and has been studied as a plasma biomarker for acute kidney injury in pediatric and adult cardiac surgery patients.[4,36–40] Our finding of no difference between groups in changes of plasma NGAL levels is of uncertain significance. Three small studies report conflicting data on the performance of plasma NGAL values during the first 6 h after cardiac surgery in adults and show that urinary NGAL levels provide a better marker of acute kidney injury.[4,36,37]

This study has certain limitations. Rather than reflecting attenuation of acute kidney injury in the preconditioning group, the observed difference in the primary outcome may indicate the protective effect of remote ischemic preconditioning on ischemic myocardium and, consequently, preserved renal plasma flow and glomerular filtration. The comparable use of vasoactive medications between groups in the initial 48 h after surgery, however, suggests that this is not the case. Studies showing associations between acute kidney injury not requiring renal replacement therapy and adverse clinical outcomes have used serum creatinine concentrations to identify episodes of acute kidney injury and have not differentiated prerenal conditions from true kidney injury.[5–10,28–30] Acute increases in serum creatinine levels because of hemodynamic effects may therefore predict poor outcomes.

Another limitation of this study is that our definition of acute kidney injury for the primary end point, and the AKIN criteria from which it was derived, do not take into consideration the duration of elevation in serum creatinine. Although even transient elevations in serum creatinine predict adverse outcomes,[41] a retrospective study of patients who underwent cardiac surgery found that duration of postoperative acute kidney injury was directly proportional

to long-term mortality.[42] We therefore assessed for differences between study groups in sustained acute kidney injury. Although the available data were limited (see Materials and Methods and Supplementary Tables S1 and S2 online), we found a lower incidence of sustained acute kidney injury in the remote ischemic preconditioning group.

Because the urinary catheters of many patients are removed within the first day after surgery at our institution, rather than employing the full AKIN definition of acute kidney injury for the primary outcome, we chose to include the criterion for urine output as a secondary outcome for the initial postoperative 12 h. Whether oliguria is an independent marker for acute kidney injury remains in doubt,[12] and we consider this a minor limitation.

Only patients and those performing creatinine and NGAL assays were blinded to randomization. It was impracticable to keep surgical and anesthesiologic staff unaware of group assignment without undue disruption of standard clinical care in the operating room. Care providers' awareness of study group assignment, therefore, could have influenced intraoperative and postoperative care and affected the outcomes of interest for this study. Although we observed comparable use of vasoactive medications in the postoperative period, we did not compare volumes of administered intravenous fluids between groups, a potential source of bias.

Our study protocol allowed for assessment of acute kidney injury only when its onset was within the first 48 h after surgery. We might have observed more acute kidney injury had we measured serum creatinine beyond 48 h for all patients, but we consider this a minor shortcoming as it is unlikely to have biased the results in favor of either group. There was a notable imbalance between study groups for previous heart surgery, a known risk factor for acute kidney injury after heart surgery,[13,43] favoring the remote ischemic preconditioning group. Nevertheless, propensity scores were not significantly different because of small imbalances in insulin-requiring diabetes mellitus, chronic obstructive pulmonary disease, and low left ventricular ejection fraction favoring the control group. Finally, the observed effect of remote ischemic preconditioning on the incidence of acute kidney injury in our study was rather large, yet it was consistent with the results of the studies of Ali *et al.*[21] and Venugopal *et al.*,[25] providing some reassurance that our findings approximate the true effect size for our study population.

Although acute kidney injury is associated with poor outcomes, it remains to be seen whether preventing acute kidney injury in any setting will reduce morbidity, mortality, and utilization of resources. A larger, multicenter study of remote ischemic preconditioning powered for these end points is warranted.

In conclusion, our study shows that remote ischemic preconditioning prevents acute kidney injury after cardiac surgery with cardiopulmonary bypass. Larger trials are necessary to determine whether this will translate into improvement in morbidity, mortality, and utilization of resources.

MATERIALS AND METHODS

Study patients

Between November 2008 and October 2009, patients who were scheduled to undergo elective cardiac surgery at Maine Medical Center and for whom cardiopulmonary bypass was anticipated were considered for enrollment. Exclusion criteria included age >80 years or <18 years, known peripheral vascular disease of the lower extremities associated with active skin necrosis or infection, end-stage renal disease, planned off-pump surgery, or inability to give informed consent. The study coordinator and two assistants identified potential subjects from the operating room schedule, enrolled consenting subjects, and assigned participants to study groups. This study was conducted in accordance with the Declaration of Helsinki Principles. The institutional review board of Maine Medical Center approved the study, and all subjects gave written informed consent before their participation. The study was registered at clinicaltrials.gov (NIH identifier NCT00821522).

Study design

Patients were stratified by type of scheduled surgery (that is, CABG, valvular surgery, combined CABG and valvular surgery, or other cardiothoracic surgery requiring cardiopulmonary bypass), and they were randomized within strata to remote ischemic preconditioning or control groups in a 1:1 ratio using manually shuffled blocks of 10, generated by the study coordinator. The allocation sequence was concealed until study groups were assigned. The study coordinator opened the opaque, sealed envelope containing the patient's group assignment before the patient arriving at the operating room.

For patients randomized to remote ischemic preconditioning, a tourniquet cuff (Stryker, Kalamazoo, MI) was placed on one thigh after the induction of anesthesia. The cuff was inflated 3 times to a pressure of 200 mm Hg for 5 min with 5-min intervals of reperfusion between inflations. The remote ischemic preconditioning regimen was conducted while patient monitors, intravascular catheters, and an indwelling bladder catheter were being placed in order not to affect the time from induction of anesthesia to initial incision compared with the control group. Tourniquet cuffs were not applied on control patients. All patients otherwise received standard intraoperative and perioperative care as directed by the cardiac surgery and cardiac anesthesiology staff, and all received isoflurane as part of the anesthetic.

Plasma samples for NGAL, a proposed biomarker for acute kidney injury, were drawn immediately before the remote ischemic preconditioning regimen and again 3 h after cardiopulmonary bypass. Serum samples for creatinine were drawn <24 h before surgery and again within the first and second postoperative 24-h periods. The concentration of NGAL was measured by fluorescence-based immunoassay (Triage NGAL, Biosite, San Diego, CA), and levels of serum creatinine were measured by enzymatic colorimetric assay (Cobas 6000, Roche, Basel, Switzerland). Patients were blinded to group assignment, as were people performing creatinine and NGAL assays.

Trial outcomes

The primary outcome was acute kidney injury, defined as any postoperative increase in the serum creatinine level of $\geqslant 0.3$ mg/dl above the preoperative value, or $\geqslant 50\%$ increase from the preoperative

value, occurring within 48 h after surgery (that is, creatinine-based criterion of AKIN). Secondary outcomes were oliguria, defined as urine output <0.5 ml/kg/h for >6 h during the initial 12 h after surgery and change in plasma NGAL levels from baseline to 3 h after cardiopulmonary bypass.

In *post hoc* analysis we assessed acute kidney injury sustained for $\geqslant 2$ and $\geqslant 3$ consecutive days. For this purpose, serum creatinine had to be $\geqslant 0.3$ mg/dl above the preoperative value on each of the consecutive days. We retrospectively collected serum creatinine data through postoperative day 5 when available from the clinical laboratory. Whereas there were insufficient data to analyze acute kidney injury with onset >48 h after surgery, among patients with acute kidney injury occurring within 48 h of surgery there were continuous data through postoperative days 3 and 4 for 89% and 79% of control patients and 83% and 67% of preconditioned patients, respectively (see Supplementary Tables S1 and S2 online).

We assessed acute kidney injury, according to AKIN stages I–III, occurring within the first postoperative 48 h and using creatinine criteria only; that is, oliguria was not considered. AKIN stage I represents an increase in serum creatinine $\geqslant 0.3$ mg/dl or an increase to 150% up to 200% of baseline; stage II, an increase to above 200% up to 300% of baseline; stage III, an increase to above 300% of baseline. We also assessed differences in the need for continuous or intermittent renal replacement therapy during the index hospitalization, the use of inotropic or vasopressor drugs at any time within 48 h after surgery and at 48 h, the need for mechanical ventilation for >48 h, reintubation, postoperative myocardial infarction or congestive heart failure, intensive care unit stay for >3 days, length of hospital stay after surgery, and death in the hospital.

Data collection

Baseline demographic and clinical data were obtained from hospital clinical records. We recorded the following comorbidities: history of diabetes mellitus, recent use of insulin before admission, history of hypertension, peripheral vascular disease, congestive heart failure, and chronic obstructive pulmonary disease. We also recorded history of previous cardiac surgery, date of most recent cardiac catheterization, left ventricular ejection fraction obtained by echocardiography or contrast ventriculography, and use of an intraaortic balloon pump <2 weeks before surgery.

For each patient, we calculated a propensity score (scale of 0 to 17 points) based on gender, congestive heart failure, ejection fraction, preoperative intraaortic balloon pump, chronic obstructive pulmonary disease, insulin-requiring diabetes, previous cardiac surgery, type of surgery, and preoperative creatinine. The propensity score has been validated for predicting acute kidney injury requiring renal replacement therapy after cardiac surgery.[43]

We recorded any use of angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, nonsteroidal anti-inflammatory drugs (including cyclooxygenase 2 inhibitors), and sulfonylureas during the 48 h before surgery. Hourly urine output was recorded for the first 12 h after surgery, but recording beyond this point was not feasible because the indwelling urinary catheters of most patients were discontinued within the first 2 postoperative days. Type of surgery, cardiopulmonary bypass time, aortic cross-clamp time, and types of inhaled anesthetics were obtained from the operative report, anesthesia record, and cardiopulmonary bypass record. Postoperative interventions, complications, and length of intensive care unit stay were obtained from a database maintained by the Cardiac Surgery Division of Maine Medical Center.

Statistical analysis

We compared baseline data and differences in outcomes between groups using the χ^2 test with Yates' correction for continuity (or Fisher's exact test when the expected value was <5) for categorical variables. Because acute kidney injury propensity scores, changes in plasma NGAL, and lengths of hospital stay were not normally distributed based on the χ^2 goodness-of-fit test, the Mann–Whitney *U* test was used for their comparisons between the two treatment groups. All other differences between groups were assessed using the unpaired *t*-test. We did not impute missing data.

For the primary analysis, we analyzed all end points on an intention-to-treat basis, and we conducted a per-protocol analysis of all renal outcomes. All reported *P*-values are two sided and unadjusted for multiple comparisons. Statistical testing was performed with SAS software, versions 9.1.3 and 9.2 (SAS Institute, Cary, NC) and GraphPad QuickCalcs (GraphPad Software, La Jolla, CA). Outcomes assessors were not blinded to group assignment. Because of an imbalance in baseline characteristics despite randomization, we performed *post hoc* analysis of the primary outcome using a Mantel–Haenzel χ^2 test stratified for previous cardiac surgery.

An interim analysis of this study was presented at the annual meeting of the American Society of Nephrology in October 2009.[44] We did not employ an α-spending function because we would not terminate the study or publish results, regardless of statistical significance, until full enrollment of 120 patients as planned.

A review of 100 consecutive patients who underwent cardiac surgery with cardiopulmonary bypass at Maine Medical Center in April and May 2008 revealed that 42% met the primary end point of the currently reported study. We therefore estimated that enrollment of 120 subjects would provide statistical power of 62% for detecting a 50% reduction of the primary end point.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. Serum creatinine levels (mg/dl) for patients with acute kidney injury—control group.

Table S2. Serum creatinine levels (mg/dl) for patients with acute kidney injury—preconditioned group.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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