The Devil Is in the Detail

Remote Ischemic Preconditioning for Perioperative Kidney Protection

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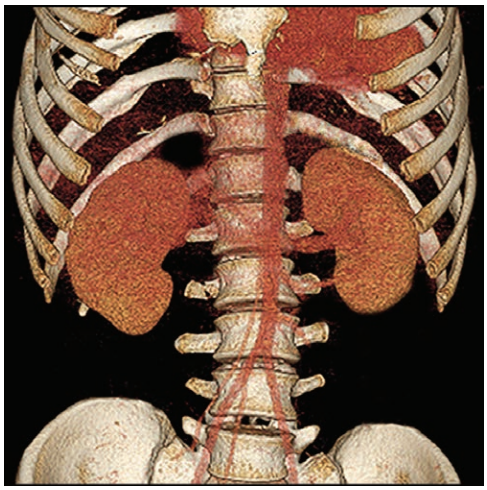
ACUTE kidney injury (AKI) is a leading postoperative complication and is associated with higher mortality and higher morbidity.[1] Even minor postoperative creatinine increases below AKI criteria are associated with adverse outcome in both noncardiac surgery[2] and cardiac surgery patients.[3] Hence, a method for the effective prevention of AKI is important and will eventually lead to the improvement of postoperative outcome. In the past, a variety of pharmacologic agents have been trialed for perioperative renoprotection (*e.g.*, fenoldepam, statins, human atrial natriuretic peptide, and nesiritide) but without conclusive evidence supporting their use.[1] In this issue of Anesthesiology, Zarbock *et al.*[4] present data on the long-term renoprotective effect of remote ischemic preconditioning (RIPC). The authors show that RIPC significantly reduced major adverse kidney events at 90 days after cardiac surgery in patients at high risk for AKI. The results of this follow-up analysis of the effects of remote ischemic preconditioning on kidney injury in high-risk cardiac surgery patients (RenalRIP) trial deliver strong evidence that RIPC provides additional long-term kidney protection. In the primary analysis of their trial, Zarbock *et al.*[5] had demonstrated RIPC to deliver short-term postoperative kidney protection: RIPC significantly reduced the rate of AKI and the use of renal replacement therapy compared to no ischemic preconditioning. RIPC could therefore be a promising method for protecting the kidney from ischemia-reperfusion injury.



"…the evidence on remote ischemic preconditioning and kidney protection is still inconclusive.…A likely reason...could be that the [most effective] exact conditions…are difficult to identify in humans."

RIPC is an experimental therapeutic strategy to protect organs against the harmful effects of ischemia-reperfusion injury by beforehand applying cycles of brief, nondetrimental ischemia and consecutive reperfusion in a distant organ (fig. 1).[6] Kharbanda *et al.*[7] were among the first to describe a noninvasive approach that was later translated to clinical use: by applying short cycles of ischemia and reperfusion to a skeletal muscle—conducted by simply inflating and deflating a standard blood pressure cuff placed on the leg—the researchers could reduce subsequently induced myocardial infarction size in pigs. Cheung *et al.*[8] subsequently demonstrated the clinical application in a proof-of-concept study in humans: the authors reported that RIPC (four 5-min cuff inflations and deflations on the thigh to 15 mmHg above systolic blood pressure) before cardiac surgery in 37 children reduced perioperative myocardial injury by less troponin I release, lowered inotrope requirements, and reduced airway pressure.

Researchers in the field have since been working on the elucidation of the underlying pathways. The stimulation with cycles of ischemia and reperfusion ultimately leads to transcriptional responses, such as the stabilization of hypoxia-induced factors (HIFs): HIF1A and HIF2A.[9,10] These changes are then signaled to other organs *via* blood-borne factors in humoral pathways. Candidates to mediate distant organ protection could potentially include soluble mediators as adenosine,

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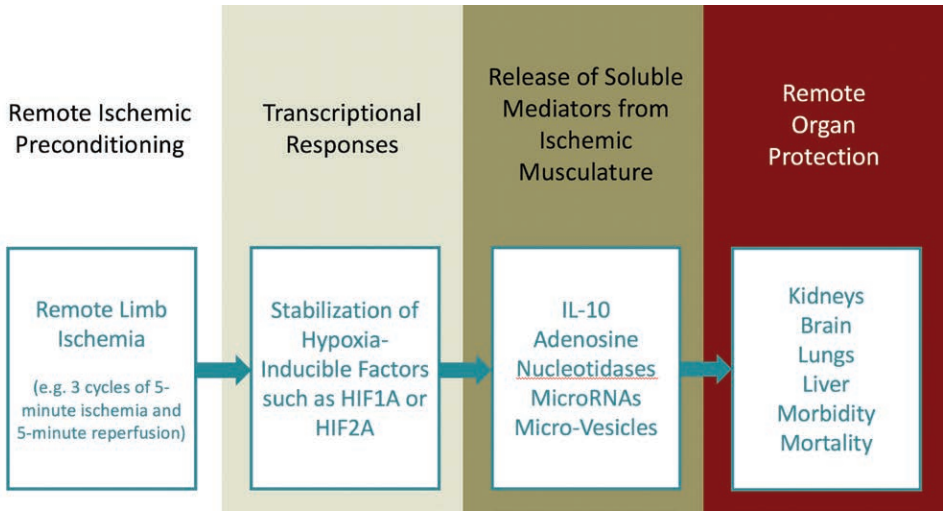

Fig. 1. Remote ischemic preconditioning (RIPC) represents an experimental approach to provide organ protection. Mechanistically, short cycles of nondetrimental ischemia and reperfusion are applied to the arm or the leg. This approach is thought to drive the stabilization of transcription factors such as hypoxia inducible factors (HIF; *e.g.*, HIF1A or HIF2A).[11] This transcriptional program mediates the release of soluble mediators from the ischemic musculature into the systemic circulation. Such mediators could potentially include cytokines (*e.g.*, interleukin [IL]-10), adenosine, circulating nucleotidases, micro-RNAs, or microvesicles. Signaling effects of these soluble mediators on remote organs such as the heart or the kidneys could then provide remote organ protection. In the current issue of Anesthesiology, Zarbock *et al*.[4] show that RIPC provides long-term kidney protection by reducing persistent renal dysfunction and renal replacement therapy dependence in cardiac surgery patients at high risk for acute kidney injury.

soluable nucleotidases, interleukin-10, micro-RNAs, or microvesicles, leading to the activation of a protective intracellular signal transduction cascade in the target organ.[6,11–13] By this means, RIPC attenuates the detrimental effects of an upcoming ischemic event in distant organs such as the heart, the lungs, the liver, or the kidneys and therefore may eventually reduce not only organ injury but also morbidity and mortality.

In this issue, Zarbock *et al*.[4] present the follow-up results of their randomized controlled clinical RenalRIP trial.[5] The multicenter, double-blinded trial demonstrated short-term postoperative kidney protection by RIPC in patients undergoing cardiac surgery and at high risk for AKI. RIPC reduced the rate of AKI within the first 72 h after surgery, reduced the need for renal replacement therapy, and reduced the intensive care unit stay. In the current follow-up analysis, the authors show that RIPC also causes long-term kidney protection and the enhanced renal recovery of those patients who did have postoperative AKI. The authors could show that RIPC reduced the frequency of the composite endpoint major adverse kidney events (consisting of mortality, the need for renal replacement therapy, and persistent renal dysfunction) at 90 days after surgery. When analyzing the components of major adverse kidney event at 90 days, RIPC significantly reduced persistent renal dysfunction (absolute risk reduction of 13%) and renal replacement therapy dependence (absolute risk reduction 7%) at 90 days after surgery but did not influence mortality. Intriguingly, of those patients who did develop AKI within 72 h after cardiac surgery, fewer suffered from persistent renal dysfunction or dialysis dependence at day 90 if they were treated with RIPC before surgery. These results provide strong evidence supporting the concept that RIPC delivers kidney protection in patients at high risk for AKI.

Despite these promising results, the evidence on RIPC and kidney protection is still inconclusive. Two additional multicenter studies by Meybohm *et al*.[14] (remote ischemic preconditioning for heart surgery [RIPHeart] trial) as well as Hausenloy *et al*.[15] (effect of remote ischemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery [ERICCA] trial) investigated the effect of RIPC on postcardiac surgery outcome. While the Zarbock *et al.* study's primary endpoint was postoperative renal function, the primary composite endpoints of both the RIPHeart and ERICCA trials were focusing on postoperative cardiovascular complications and death. Interestingly, the results of both Meybohm *et al*.[14] and Hausenloy *et al*.[15] did not show any effect of RIPC, neither on the primary composite endpoints (as well as on any of its individual components), nor on the secondary endpoints. In particular regarding postcardiac surgery renal function, both trials did not show any renoprotective effects for RIPC (postoperative renal function was a secondary endpoint in both the RIPHeart and the ERICCA trials), contrasting the results of RenalRIP. The differences in the results may be explained by the different patient populations. The RenalRIP trial included only high-risk patients, while both the RIPHeart and ERICCA trials included low-risk patients.

A likely reason for the contradicting results could be that the exact conditions for the most effective RIPC are difficult to identify in humans. Animal studies have revealed that this is

in fact a challenging task. For example, an experimental study designed to define optimal conditions for myocardial ischemic preconditioning in mice examined numerous different preconditioning regimens. Protocol optimization in this study included different cycle numbers, body temperatures, ischemia times, *etc*., before the authors were able to identify a regimen that reliably produced organ protection.[16] Both the RIPHeart and ERICCA trials used a sequence of four times 5-min ischemia, with 5 min of reperfusion in-between, whereas the RenalRIP protocol only used three times 5 min of ischemia with identical reperfusion intervals. It may very well be that the devil is in the detail and the optimal protocol for effective RIPC in humans has not yet been discovered. Systematic evaluation of RIPC protocols in humans is needed to find the optimal one for postoperative organ protection. Such studies could initially be done in volunteers to examine optimal release of soluble mediators, such as interleukin-10,[11] before examining organ protection in patients. As the RIPC protocol of the current study by Zarbock *et al*.[4] provided robust protection, it will also be critical to repeat their findings in larger patient populations and different surgical and patient settings.

In summary, the exciting finding of Zarbock *et al*.[4] demonstrate for the first time that RIPC also has long-term renoprotective effects in high-risk surgical patients. These impressive data are the first step toward clinical implementation of RIPC for kidney protection. However, since this was a relatively small study presenting a large effect size, the findings need to be confirmed in large-scale multicenter trials. It will be exciting to see this field further evolve with the hope that in the near future, RIPC may become a routine clinical strategy to provide kidney protection for surgical patients.

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Competing Interests

The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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Long-term Effects of Remote Ischemic Preconditioning on Kidney Function in High-risk Cardiac Surgery Patients

Follow-up Results from the RenalRIP Trial

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ABSTRACT

Background: In a multicenter, randomized trial, the authors enrolled patients at high-risk for acute kidney injury as identified by a Cleveland Clinic Foundation score of 6 or more. The authors enrolled 240 patients at four hospitals and randomized them to remote ischemic preconditioning or control. The authors found that remote ischemic preconditioning reduced acute kidney injury in high-risk patients undergoing cardiac surgery. The authors now report on the effects of remote ischemic preconditioning on 90-day outcomes.

Methods: In this follow-up study of the RenalRIP trial, the authors examined the effect of remote ischemic preconditioning on the composite endpoint major adverse kidney events consisting of mortality, need for renal replacement therapy, and persistent renal dysfunction at 90 days. Secondary outcomes were persistent renal dysfunction and dialysis dependence in patients with acute kidney injury.

Results: Remote ischemic preconditioning significantly reduced the occurrence of major adverse kidney events at 90 days (17 of 120 [14.2%]) *versus* control (30 of 120 [25.0%]; absolute risk reduction, 10.8%; 95% CI, 0.9 to 20.8%; $P = 0.034$). In those patients who developed acute kidney injury after cardiac surgery, 2 of 38 subjects in the remote ischemic preconditioning group (5.3%) and 13 of 56 subjects in the control group (23.2%) failed to recover renal function at 90 days (absolute risk reduction, 17.9%; 95% CI, 4.8 to 31.1%; $P = 0.020$). Acute kidney injury biomarkers were also increased in patients reaching the major adverse kidney event endpoint compared to patients who did not.

Conclusions: Remote ischemic preconditioning significantly reduced the 3-month incidence of a composite endpoint major adverse kidney events consisting of mortality, need for renal replacement therapy, and persistent renal dysfunction in high-risk patients undergoing cardiac surgery. Furthermore, remote ischemic preconditioning enhanced renal recovery in patients with acute kidney injury. **(ANESTHESIOLOGY 2017; 126:00-00)**

IN cardiac surgery patients, acute kidney injury (AKI) is associated with an increased risk of short-term adverse outcomes.[1,2] However, longer-term outcomes for patients with AKI need further attention.[3,4] Moreover, interventions that reduce AKI after cardiac surgery may not impact long-term outcomes.[5] Thus, after an episode of AKI, the risks of mortality and of subsequent chronic kidney disease with or without the need for renal replacement therapy remain uncertain. A recently published meta-analysis by Coca *et al.*[6] reported absolute rates of chronic kidney disease after AKI approximately 50% higher than that of mortality, but the analysis was limited due to high statistical heterogeneity.

The effect of remote ischemic preconditioning (RIPC) on renal function after cardiac surgery has been investigated in the last years, offering conflicting results.[7–9] We have recently

What We Already Know about This Topic

- Previous studies have demonstrated that acute kidney injury is associated with an increased risk of short-term adverse outcomes after cardiac surgery
- This study is a follow-up study from the RenalRIP cohort to determine the effects of remote ischemic preconditioning on the 90-day composite endpoint major adverse kidney events consisting of all-cause mortality, the receipt of renal replacement therapy, and persistent renal dysfunction without dialysis

What This Article Tells Us That Is New

- Remote ischemic preconditioning significantly reduced the 3-month incidence of a composite endpoint major adverse kidney events consisting of mortality, need for renal replacement therapy, and persistent renal dysfunction in high-risk patients undergoing cardiac surgery

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published the results of a multicenter, randomized controlled trial investigating the effects of RIPC on the occurrence of AKI in high-risk patients undergoing cardiac surgery and demonstrated that this intervention significantly reduced the rate of AKI and need for renal replacement therapy.[9] Moreover, we have shown that the effectiveness of this intervention was strongly associated with the release of cell cycle arrest biomarkers into the urine. Patients who responded to RIPC with an increase in urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7; [TIMP-2]·[IGFBP7]) greater than or equal to 0.5 $(ng/ml)^2/1{,}000$ before surgery had a significantly reduced rate of AKI compared to patients with lower urinary [TIMP-2]·[IGFBP7]. These same biomarkers predicted AKI when they increased as a result of surgery, as shown previously.[10–12] However, longer-term outcomes of patients treated with RIPC are unknown.

Here, we report a follow-up study from the RenalRIP cohort to determine the effects of RIPC on the 90-day composite endpoint major adverse kidney events (MAKE) consisting of all-cause mortality, the receipt of renal replacement therapy, and persistent renal dysfunction without dialysis. Documentation of long-term effects of RIPC on renal recovery and other outcomes is important for understanding the biology of this intervention and for patient care.

Materials and Methods

The RenalRIP trial has been described in detail elsewhere.[9] Briefly, 240 patients at high risk for AKI who underwent cardiac surgery with the use of cardiopulmonary bypass (CPB) were enrolled at four sites in Germany from August 2013 to June 2014. We used a Cleveland Clinic Foundation score of 6 or more to define patients at high risk for AKI.[13] The score is composed of different risk factors, including patient characteristics, comorbidities, and type of surgery. Patients were randomized on a 1:1 basis stratified by center. On the day of surgery, patients were assigned to undergo either RIPC or sham-RIPC (control) and the intervention was provided by an investigator not involved in the care of the patient. Patients, anesthesiologists and staff providing care of the patient, cardiac surgeons, and intensive care physicians were blinded for treatment assignment. For induction of anesthesia, all patients received sufentanil with either benzodiazepines (Bochum, Freiburg, and Tübingen; 100% in these centers) alone or in combination with barbiturates (Münster; 100% in this center). To maintain anesthesia, a combination of sufentanil and volatile anesthetics was used (sufentanil: all centers, 100% in these centers; isoflurane: Bochum, 100% in this center; sevoflurane: Freiburg, Münster, and Tübingen, 100% in these centers). Propofol was not used due to potential interference with RIPC.[14] None of the patients received regional anesthesia. After induction of anesthesia and before skin incision, we performed RIPC consisting of three cycles of 5-min inflation of a blood pressure cuff to 200 mmHg (or at least to a pressure 50 mmHg higher than the systolic arterial pressure) to one upper arm followed by 5-min reperfusion with the cuff deflated. In patients assigned to the control group, sham-RIPC intervention was induced by three cycles of upper-limb pseudo-ischemia (*low pressure*: 5-min blood pressure cuff inflation to a pressure of 20 mmHg and 5-min cuff deflation). The surgical procedure and perioperative care were performed according to the standard at each center. According to the recommendations of the American College of Cardiology Foundation (Washington, D.C.) and the Kidney Disease: Improving Global Outcomes (KDIGO; Brussels, Belgium) guidelines, angiotensin-converting enzyme inhibitors (ACEi) and angiotensin-II receptor blockers (ARBs) were discontinued before surgery. Medication was reconvened once the patient was hemodynamically stable. The RenalRIP trial was approved by the institutional review board at each site. All subjects (or legally authorized representatives) provided written informed consent. The trial is registered at http://www.drks.de (identifier: DRKS00005333; principal investigator: Dr. Zarbock; registration date: July 11, 2013).

Sample and Data Collection

Blood samples were collected by standard methods before surgery and at prespecified time points after surgery for measurement of serum creatinine concentrations (4 h after cardiac surgery and on every morning for at least 3 days after cardiac surgery). Urine samples for biomarkers were collected before RIPC/sham-RIPC, after inducing RIPC/sham-RIPC, and at 4, 12, and 24 h after surgery. The samples were centrifuged, and urine supernatants and serum were frozen within 2 h after collection and thawed immediately before analysis. All clinical data, including patient demographics, need for renal replacement therapy, length of stay in the intensive care unit, length of stay in the hospital, 30- and 90-day mortality, previous health history, serum creatinine, concentrations of various biomarkers, and hourly urine output, were collected and stored in a password-protected data set.

Clinical Endpoints

The key endpoint of this follow-up analysis MAKE consisting of the composite of death, dialysis, or persistent renal dysfunction at day 90 was determined from hospital records or for patients discharged alive and not on dialysis, telephone calls to the general practitioner, the subject or family members at 3 months after enrollment. The individual components were defined as follows: if the patient died, the mortality endpoint was met but not dialysis dependency or persistent renal dysfunction. If the patient met the dialysis endpoint, the subject was also defined as persistent renal dysfunction. If the persistent renal dysfunction endpoint was met but not the dialysis endpoint, then only persistent renal dysfunction was met. If the subject was determined to have died or been on dialysis at the time of telephone assessment, the date of death or dialysis was recorded. We defined persistent renal dysfunction as serum creatinine levels greater than or equal to 0.5 mg/dl higher than baseline serum creatinine[15,16] in

patients not receiving dialysis or dialysis dependency. Patients who died within 90 days could not be evaluated for persistent renal dysfunction. This composite endpoint has been recommended because death is a competing endpoint otherwise.[17] AKI status during the first 72 h after enrollment was classified using the KDIGO guidelines on the basis of serum creatinine and urine output.[18] The reference values for serum creatinine were obtained as described previously.[9]

Laboratory Methods

As described previously, urinary TIMP-2 and IGFBP7 were analyzed by investigators not involved in the care of the patient and blinded to clinical data using a clinical immunoassay (NephroCheck Test and ASTUTE140 Meter; Astute Medical, USA).[9] The ASTUTE140 Meter automatically multiplies the concentrations of the two biomarkers together and divides this product by 1,000 to report a single numerical test result with units of (nanograms per milliliter)2/1,000 (the units for all [TIMP-2]·[IGFBP7] test values in this report). Urine neutrophil gelatinase–associated lipocalin (NGAL) was measured with a commercially available assay (Dianova, Germany) according to the manufacturer's protocol.

Statistical Analysis

For the parental analysis, we calculated a necessary sample size based on the primary endpoint (occurrence of AKI within 72 h after cardiac surgery) using nQuery Advisor software (Statistical Solutions; version 7). The primary efficacy analysis was intended to show superiority of RIPC in high-risk cardiac surgery patients, applying a two-sided chi-square test on significance level α=0.05. Based on an observational study, we performed in a similar patient population[12] the expected AKI rate in the control group treated with sham-RIPC was 50%. The expected absolute risk reduction (ARR) for AKI was 18% based on a published single-center study investigating the effect of RIPC on AKI after cardiac surgery.[19] Resulting from these considerations and a power of 80%, the required sample size was calculated to be 117 evaluable patients per treatment group, *i.e.*, 234 in total. An additional six patients were recruited in order to account for loss to follow-up or nonevaluable data.

Here, we describe the statistical methods that were selected to analyze the secondary outcomes of the RenalRIP trial (preplanned analyses) and the composite outcome variable MAKE at day 90. Continuous variables are described by mean ± SD in case of normally distributed data and as median (Q1 to Q3) in case of skewed data. Categorical variables are described by absolute and relative frequencies. Differences between groups are reported as ARR and its corresponding 95% CI. The key outcome parameter of this analysis (MAKE at day 90) as well as the other secondary outcomes (persistent renal dysfunction, dialysis, and mortality) were analyzed by chi-square test to test for association with the treatment group (RIPC *vs.* Sham-RIPC). If assumptions for the chi-square test were not fulfilled, Fisher exact test was applied. For theses analyses, odds ratios (OR) and 95% CI are reported. To assess the sensitivity of the results, we defined persistent renal dysfunction as serum creatinine increase of 50% or more compared to baseline value or dialysis dependency. Additionally, to evaluate the treatment effect under consideration of the recruiting centers, we applied the Cochran–Mantel–Haenszel (CMH) test. Mortality was also analyzed by time-to-event methods, *i.e.*, Kaplan–Meier plots and Cox proportional hazards model.

For all outcomes, adjusted ORs and corresponding 95% CIs were estimated in a logistic regression model containing the treatment group, age, gender, and chronic kidney disease status. The variables examined in the regression model were selected because these are known to be associated with the development of AKI.[13] Biomarker measurements were analyzed at each time point individually using Mann–Whitney U tests to compare the treatment groups. Receiver operator characteristic (ROC) curve analysis was applied to assess a biomarker's predictive performance with respect to MAKE prediction at day 90. As a result, the area under the ROC curve (AUC) and its 95% CI were reported. AUCs were tested against the null hypothesis H_0: AUC = 0.5. Cutpoints for selected time points were determined by maximizing the Youden index: max (sensitivity + specificity – 1).[20,21]

For all statistical tests, a significance level of 5% was assumed and no correction for the multiple testing problem was applied. The results are thus interpreted as to generate new hypotheses. Analyses were performed using SPSS 22 (IBM Corp., USA, Released 2013; IBM SPSS Statistics for Windows, version 22.0) and the SAS software 9.4 (SAS Institute Inc., USA).

Results

In total, 240 subjects were enrolled in the RenalRIP study, and for this analysis, no subjects were excluded as illustrated in figure 1. In our previous article,[9] we have shown the demographic and operative characteristics of the patients in the control and intervention groups. Table 1 describes the demographic and operative data of MAKE-positive patients *versus* MAKE-negative patients. Full 3-month outcome was known for 240 (100% of the initial cohort) patients, with 47 (19.6%) patients meeting the endpoint of MAKE and 193 (80.4%) patients not meeting the endpoint MAKE at day 90. Patients with MAKE at day 90 were more likely to be older (P = 0.002); the number of patients with congestive heart failure and ACEi or ARBs was higher in the group (P = 0.018 and P = 0.024, respectively) and showed higher rates of major postoperative bleeding (P = 0.009). In addition, appendix A1 shows the demographic and operative data stratified by Sham-RIPC and RIPC.

RIPC significantly reduced the occurrence of MAKE at day 90 (17 of 120 [14.2%]) compared with Sham-RIPC (30 of 120 [25.0%]; ARR, 10.8%; 95% CI, 0.9 to 20.8%; P = 0.034; table 2). Considering the different components of the composite endpoint, persistent renal dysfunction (ARR, 12.7%;

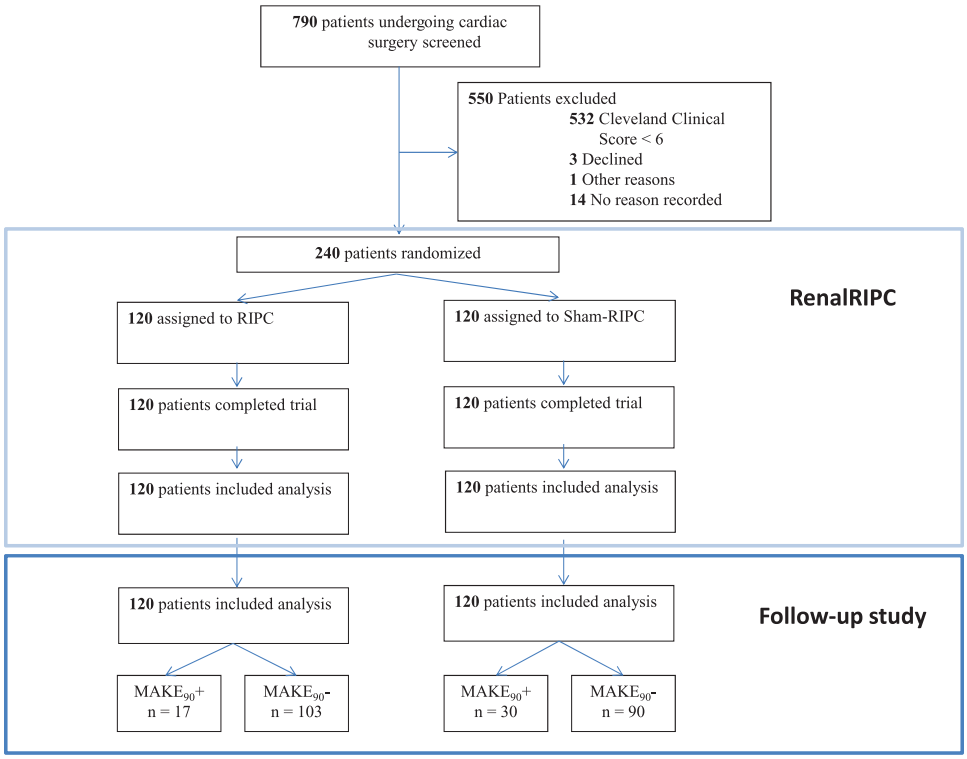

Fig. 1. Patient enrollment and allocation to the remote ischemic preconditioning (RIPC) and control (Sham-RIPC) arms. The first part (*light blue box*) shows the recruitment of the initial RenalRIP trial,[9] while the second part (*dark blue box*) shows the analyzed cohort of this follow-up analysis. $MAKE_{90}^{+}$ = patients meeting the endpoint major adverse kidney events; $MAKE_{90}^{-}$ = patients not meeting the endpoint major adverse kidney events.

OR, 0.262; 95% CI, 0.101 to 0.681; $P = 0.004$; table 2) and renal replacement therapy (ARR, 7.2%; OR, 0.311; 95% CI, 0.097 to 0.997; $P = 0.040$; table 2; fig. 2A) were significantly higher in the Sham-RIPC group than in the RIPC group. Sensitivity analyses using the modified definition of persistent renal dysfunction (persistent elevation of serum creatinine greater than or equal to 50% of baseline) also demonstrated significant results (ARR, 9.8%; 95% CI, 1.40 to 18.21%; $P = 0.024$). Mortality was similar in both groups (RIPC 9.2% *vs.* Sham-RIPC 8.3%; $P = 0.819$; table 2; fig. 2B). We repeated the analysis of outcomes stratified by center and found no differences compared to the unstratified analyses (MAKE at day 90: OR, 0.502; 95% CI, 0.261 to 0964; $P_{CMH} = 0.035$; persistent renal dysfunction at day 90: OR, 0.263; 95% CI, 0.101 to 0.682; $P_{CMH} = 0.003$; renal replacement therapy at day 90: OR, 0.297; 95% CI, 0.092 to 0.961; $P_{CMH} = 0.034$) demonstrating homogeneous treatment outcome.

In the 108 of 240 (45%) patients who developed AKI after cardiac surgery (RIPC 45 [37.5%] *vs.* Sham-RIPC 63 [52.5%]), 2 of 38 subjects in the RIPC group (5.3%) and 13 of 56 subjects in the Sham-RIPC group (23.2%) failed to recover renal function by day 90 (ARR, 17.9%; 95% CI, 4.8 to 31.1%; $P = 0.020$; table 2). We observed a reduced dependence on renal replacement therapy in the RIPC group (one patient [2.6%]) compared to that in the Sham-RIPC group (eight patients [14.3%]) within 90 days after randomization (ARR, 11.7%; 95% CI, 1.2 to 22.1%; $P = 0.079$). Mortality was similar in both groups (RIPC: 15.6% *vs.* Sham-RIPC: 11.1%; $P = 0.498$).

The effect of RIPC *versus* Sham-RIPC was confirmed in a logistic regression analysis adjusted for age, gender, and chronic kidney disease (MAKE at day 90: OR, 0.505; 95% CI, 0.258 to 0.988; $P = 0.046$; persistent renal dysfunction at day 90: OR, 0.258; 95% CI, 0.098 to 0.678; $P = 0.006$; renal replacement therapy at day 90: OR, 0.306; 95% CI, 0.094 to 0.991; $P = 0.048$; and mortality at day 90: OR, 1.179; 95% CI, 0.472 to 2.948; $P = 0.724$; table 3).

As shown previously,[9] baseline urinary [TIMP-2]·[IGFBP7] tested immediately before RIPC or Sham-RIPC did not differ between the 2 groups ($P = 0.33$). Urinary [TIMP-2]·[IGFBP7] tested 4 h after CPB was significantly higher in patients meeting the endpoint (MAKE-negative patients: 0.57 $[ng/ml]^2/1{,}000$; MAKE-positive patients: 1.01 $[ng/ml]^2/1{,}000$; $P = 0.021$). A ROC analysis for MAKE at day 90 demonstrated best performance for [TIMP-2]·[IGFBP7] at 4 h (AUC, 0.641; 95% CI, 0.546 to 0.736; $P = 0.004$) and 12 h (AUC, 0.623; 95% CI, 0.515 to 0.731; $P = 0.026$) after cardiac surgery (tables 4 and 5). The same was true for urinary NGAL levels (tables 4 and 5). The AUCs for the combination of [TIMP-2]·[IGFBP7] with NGAL at the different time points were not different ($P > 0.05$). The sensitivity, specificity, Youden index, and cutoff points for the 4- and 12-h time points after cardiac surgery were calculated, as shown in tables 4 and

Table 1. Characteristics of Patient Cohort

	$MAKE_{90}^{+}$ (n = 47)	$MAKE_{90}^{-}$ (n = 193)	*P* Value
Age, yr, mean (SD)	73.9 (7.8)	69.5 (9.7)	0.002
Male, n (%)	28 (59.6)	123 (63.7)	0.597
ASA grade, n (%)			0.321
1	0 (0)	0 (0)	
2	11 (23.4)	40 (20.7)	
3	31 (66.0)	143 (74.1)	
4	5 (10.6)	10 (5.2)	
CCF score, median (Q1–Q3), points*	6 (6–6)	6 (6–6)	0.551
Preoperative creatinine, mean (SD), mg/dl	1.3 (0.5)	1.1 (0.4)	0.076
eGFR, mean (SD), ml/min per 1.73 m^2	52.4 (12.4)	57.6 (15.0)	0.027
Creatinine day 90, mean (SD), mg/dl	1.7 (0.8)	1.1 (0.4)	< 0.001
Comorbidities, n (%)			
Hypertension	46 (97.9)	186 (96.4)	1†
Congestive heart failure	45 (95.7)	158 (81.9)	0.018
Diabetes	18 (38.3)	72 (37.3)	0.900
COPD	14 (29.8)	62 (32.1)	0.757
Chronic kidney disease	19 (40.4)	55 (28.5)	0.112
Previous heart surgery	7 (14.9)	20 (10.4)	0.378
Medication, n (%)			
Aspirin	27 (57.4)	116 (60.1)	0.739
Clopidogrel	4 (8.5)	22 (11.4)	0.568
β blockers	34 (72.3)	112 (58.0)	0.072
Statins	36 (76.6)	129 (66.8)	0.196
Diuretics	30 (63.8)	104 (53.9)	0.218
ACEi or ARBs	35 (74.5)	109 (56.5)	0.024
Operative characteristics, n (%)			
Aortic cross-clamp duration, median (Q1–Q3), min	89 (63–120)	84 (60–110)	0.371
Cardiopulmonary bypass time, median (Q1–Q3), min	120 (90–175)	121 (90–178)	0.525
CABG only	15 (31.9)	65 (33.7)	0.818
Valve only	6 (12.8)	43 (22.3)	0.147
Combined or other	26 (55.3)	85 (44.0)	0.164
Postoperative complications, n (%)			
Myocardial infarction	3 (6.7)	8 (4.1)	0.468
Major bleeding	13 (28.9)	25 (13.0)	0.009
Cerebral insult	1 (2.2)	4 (2.1)	0.950
Cerebral hemorrhage	0 (0)	0 (0)	
Pulmonary embolism	0 (0)	0 (0)	

Demographic and operative data of patients meeting major adverse kidney event ($MAKE_{90}^{+}$) *versus* patients not meeting major adverse kidney event ($MAKE_{90}^{-}$) at day 90.

*Cleveland Clinic Foundation (CCF) score (0 to 17 points) is composed of 13 preoperative risk factors, comorbidities, and type of surgery. A higher number correlates with a higher rate of dialysis-dependent acute kidney injury after cardiac surgery (Thakar *et al.*[13]). †Fisher exact test.

ACEi = angiotensin-converting enzyme inhibitors; ARB = angiotensin-II receptor blockers; ASA = American Society of Anesthesiology grade (grade 5 patients were not included); CABG = coronary artery bypass graft; COPD = chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate.

5. Combining [TIMP-2]·[IGFBP7] with NGAL did not demonstrate superior predictive value for MAKE at day 90 compared to one marker alone (appendix A2).

Next, we assessed the relationship between urinary biomarker concentrations 4, 12, and 24 h after CPB and persistent renal dysfunction at day 90 (table 6). For [TIMP-2]·[IGFBP7], the AUC for the 4- and 12-h time points were 0.692 (95% CI, 0.563 to 0.821; $P = 0.004$) and 0.677 (95% CI, 0.523 to 0.831; $P = 0.024$), respectively (table 6). The AUC for the composite time point was 0.696 (95% CI, 0.555 to 0.836; $P = 0.006$). Similar results were obtained for NGAL (table 6). The ROC curves for the combination of [TIMP-2]·[IGFBP7] with NGAL at the different time points were not different ($P > 0.05$). Combining [TIMP-2]·[IGFBP7] with NGAL did not improve the precision for persistent renal dysfunction at day 90 compared to one marker alone (appendix A3).

Discussion

The results of this follow-up of the randomized controlled clinical RenalRIP trial[9] show that RIPC improves short- as well as long-term outcomes of high-risk patients undergoing cardiac surgery.

In recent years, several studies investigating the effects of RIPC have been published with variable results. In contrast to studies showing a positive effect of RIPC on the heart and kidney,[9,19,22] several studies were unable to demonstrate that RIPC

Table 2. Outcomes of Patients by the Treatment Group

	n	Sham-RIPC	RIPC	*P* Value	ARR % (95% CI)	OR/HR* (95% CI)
All patients						
$MAKE_{90}$, n (%)	120/120	30 (25.0)	17 (14.2)	0.034	10.8 (0.9–20.8)	0.495 (0.256–0.957)
PRD_{90}*, n (%)	110/109	20 (18.2)	6 (5.5)	0.004	12.7 (4.3–21.1)	0.262 (0.101–0.681)
RRT_{90}*, n (%)	110/109	12 (10.9)	4 (3.7)	0.040	7.2 (0.4–14.1)	0.311 (0.097–0.997)
$Mortality_{90}$, n (%)	120/120	10 (8.3)	11 (9.2)	0.819	−0.8 (−8.0 to 6.3)	1.127* (0.458–2.775)
28-day survival, % (95% CI)		96.6 (91.1–98.7)	94.9 (89.0–97.7)	0.795		
Length of hospital stay after surgery, median (Q1–Q3)	110/106	10 (8–16)	9 (7–14)	0.615†		
Patients with AKI						
$MAKE_{90}$, n (%)	63/45	20 (31.8)	9 (20.0)	0.175	11.8 (−4.7 to 28.1)	0.538 (0.218–1.326)
PRD_{90}*, n (%)	56/38	13 (23.2)	2 (5.3)	0.020	17.9 (4.8–31.1)	0.184 (0.039–0.869)
RRT_{90}*, n (%)	56/38	8 (14.3)	1 (2.6)	0.079‡	11.7 (1.2–22.1)	0.162 (0.019–1.354)
$Mortality_{90}$, n (%)	63/45	7 (11.1)	7 (15.6)	0.498	−4.4 (−17.6 to 8.7)	1.193* (0.401–3.550)
28-day survival, % (95% CI)		93.4 (83.5–97.5)	91.1 (78.0–96.6)	0.751		
Length of hospital stay after surgery, median (Q1–Q3)	56/38	10 (8–21)	10 (9–18)	0.841†		

Odds ratios (ORs) and hazard ratios (HRs) quantify the acute kidney injury (AKI) risk of remote ischemic preconditioning (RIPC) as compared to sham (reference).

*Excluding patients who died. †Mann–Whitney U test. ‡Fisher exact test was used due to expected counts less than 5 in the cross-table.

ARR = absolute risk reduction for RIPC; $MAKE_{90}$ = major adverse kidney events within 90 days; PRD_{90} = persistent renal dysfunction at day 90; RRT_{90} = dialysis dependence within 90 days.

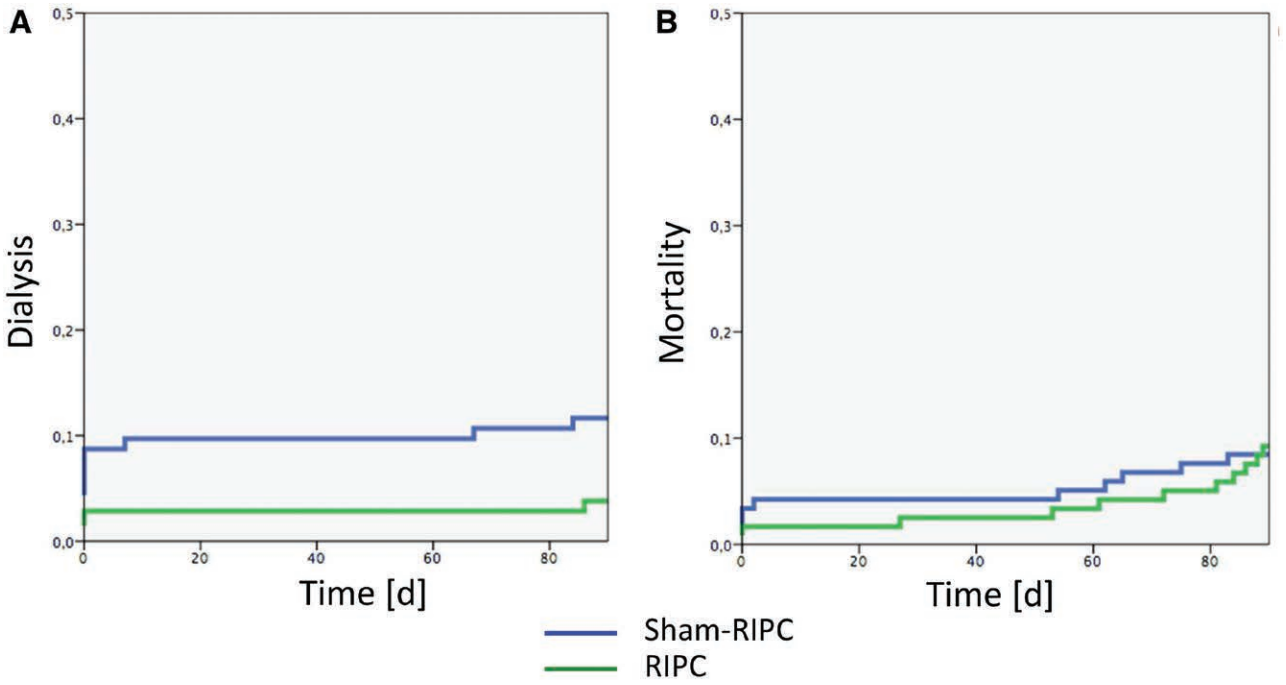

Fig. 2. Kaplan–Meier curves for dialysis (*A*) and death (*B*) within 90 days after study enrollment. Cumulative dialysis or death within 90 days for control (Sham-RIPC; *blue line*) or remote ischemic preconditioning (RIPC) patients (*green line*). (*A*) log-rank $P = 0.040$ (*B*) log-rank $P = 0.849$.

can affect organ function, complications, or mortality.[7,8,23,24] Two recently published large multicenter trials investigating more than 3,000 patients demonstrated that RIPC did not affect either composite endpoints or mortality.[7,8] These results might be explained by differences in study design. Applying RIPC in high-risk patients reduced AKI,[9] whereas the use of this same intervention in low-risk patients had no effect on myocardial infarction, occurrence of AKI, or mortality.[7,8] Another very important difference between the studies, which might explain the different results, is the anesthetic regime during surgery. It has been shown that propofol can affect the effects of RIPC.[14] In the two recently published multicenter trials, the vast majority of patients received propofol for anesthesia, which could have diminished or abrogated the effect of

Table 3. Logistic Regression Analysis

Outcome Parameter	Adjusted OR (95% CI)	*P* Value
AKI	0.547 (0.326–0.919)	**0.023**
Moderate/severe AKI	0.443 (0.227–0.868)	**0.018**
$MAKE_{90}$	0.505 (0.258–0.988)	**0.046**
PRD_{90}	0.258 (0.098–0.678)	**0.006**
RRT_{90}	0.306 (0.094–0.991)	**0.048**
$Mortality_{90}$	1.179 (0.472–2.948)	0.724
Survival	HR: 1.212 (0.488–3.008)	0.678

Bold *P* values are considered statistically significant. Adjusted odds ratios (OR) for the effect of remote ischemic preconditioning *versus* Sham on the selected outcome parameters. Adjustment has been performed for age, gender, and chronic kidney disease status.

AKI = acute kidney injury; HR = hazard ratio; $MAKE_{90}$ = major adverse kidney events within 90 days; $Mortality_{90}$ = mortality at day 90; PRD_{90} = persistent renal dysfunction at day 90; RRT_{90} = dialysis dependence within 90 days.

RIPC. In our trial, we specifically avoided propofol and found that RIPC significantly reduced the occurrence of MAKE at day 90 compared to sham. Furthermore, in patients who nevertheless developed AKI after surgery, RIPC significantly improved renal recovery as seen by lower rates of renal replacement therapy and persistent renal dysfunction.

Surgical patients commonly experience postoperative increases in creatinine levels. A recently published trial suggests that even small increases (Δcreatinine 25 to 49% above baseline but less than 0.3 mg/dl) in postoperative creatinine levels are associated with adverse outcomes.[25] This was more pronounced in noncardiac surgery patients. These results suggest that RIPC might be an effective therapeutic approach to prevent even mild forms of postoperative kidney dysfunction and to improve surgical outcomes. Moreover, one could raise the concern of ACEi and ARBs influencing the effects of RIPC. The same number of patients in the control and RIPC groups received ACEi or ARBs (60.8 *vs.* 59.2%, respectively; $P > 0.05$). ACEi and ARBs were discontinued before surgery, and according to the recommendations of the American College of Cardiology Foundation[26] and the KDIGO guidelines,[18] patients received ACEi or ARBs after they became hemodynamically stable. Due to the preoperative discontinuation of the ACEi and ARBs, it is unlikely that these drugs potentiated the effects of RIPC.

The mechanisms responsible for the benefit of RIPC are not completely understood. Recent evidence suggests that *in situ* preconditioning induces activation of cardiac hypoxia-inducible factor (HIF)-1α. *In vivo* small interfering RNA repression of cardiac HIF-1α resulted in abolished cardioprotection by ischemic preconditioning.[27] Another study demonstrated that HIF-1 activates interleukin (IL)-10 gene transcription and is required for RIPC.[28] Our recently published data suggest that RIPC induces the release of damage-associated molecular patterns from the ischemic tissue and that these molecules may engage self-protective mechanisms in the kidney such as cell cycle arrest.[9]

Biomarkers may further aid in the interpretation of results from interventional trials. Coca *et al.*[29] have recently shown that several AKI biomarkers measured in the perioperative period after cardiac surgery correlated with long-term mortality. The authors of the Translational Research Investigating Biomarker Endpoints in AKI study demonstrated that, compared with the first tertiles, the third tertiles of peak biomarker percentages of urinary kidney injury molecule-1, NGAL, liver fatty acid binding protein, IL-18, and albumin were all associated with a significantly increased risk of mortality in those subjects who developed AKI after surgery. This effect was also significant for IL-18 and kidney injury molecule-1 in patients without AKI. Both IGFBP7 and TIMP-2 are involved with the phenomenon of G_1 cell cycle arrest during the very early phases of cell injury. Importantly, both TIMP-2 and IGFBP7 may increase in response to a wide variety of insults (inflammation, oxidative stress, ultraviolet radiation, drugs, and toxins).[30–32] The results of several studies of urinary [TIMP-2]·[IGFBP7] demonstrated that these markers enable early diagnosis and risk stratification of AKI in a wide range of critically ill patients.[10,12] In terms of timing, this signal represents an early point of cellular stress. Biomarkers that can detect cellular stress may be more useful than markers of injury or cell death. These elevations are independent of the presence of other chronic conditions such as chronic kidney disease.[33] Moreover, there is growing evidence that damage markers may play an important role in the progression of AKI to maladaptive repair resulting in progression of fibrosis to chronic kidney disease.[34,35] A recently published study showed that [TIMP-2]·[IGFBP7] measured

Table 4. Receiver Operating Characteristic Analysis for Major Adverse Kidney Events at Day 90

	N	N^+	N^-	AUC (95% CI)	*P* Value	N	N^+	N^-	AUC (95% CI)	*P* Value
Urinary [TIMP-2]·[IGFBP7]										
Preintervention	237	46	191	0.592 (0.503–0.682)	0.044	232	47	185	0.585 (0.501–0.668)	0.048
Postintervention	231	46	185	0.541 (0.453–0.630)	0.363	230	46	184	0.605 (0.519–0.691)	0.017
4-h post-CPB	214	44	170	0.641 (0.546–0.736)	0.004	222	47	175	0.617 (0.522–0.713)	0.016
12-h post-CPB	230	44	186	0.623 (0.515–0.731)	0.026	227	45	182	0.618 (0.524–0.713)	0.014
24-h post-CPB	230	45	185	0.539 (0.433–0.645)	0.476	224	46	178	0.585 (0.493–0.677)	0.070
Composite	238	46	192	0.617 (0.513–0.721)	0.028	232	47	185	0.639 (0.547–0.731)	0.003

AUC = area under the receiver operator characteristics curve; CPB = cardiopulmonary bypass; IGFBP7 = insulin-like growth factor-binding protein 7; N = all patients; N^+ = patients with positive major adverse kidney events at day 90; N^- = patients with negative major adverse kidney events at day 90; TIMP-2 = tissue inhibitor of metalloproteinases-2.

Table 5. Evaluation of Cutoff Values for Urinary [TIMP-2]·[IGFBP7] and Urinary NGAL

	Urinary [TIMP-2]·[IGFBP7]				Urinary NGAL			
Cutoff Values	Cutoff	Youden Index	Sensitivity	Specificity	Cutoff	Youden Index	Sensitivity	Specificity
Preintervention	0.38	0.20	0.78	0.41	4.67	0.22	0.94	0.28
Postintervention	0.83	0.14	0.87	0.27	4.09	0.23	0.94	0.30
4-h post-CPB	0.36	0.28	0.57	0.71	51.95	0.22	0.55	0.66
12-h post-CPB	0.68	0.32	0.50	0.82	15.24	0.26	0.67	0.59
Composite	0.86	0.29	0.54	0.75	483.81	0.24	0.34	0.90

CPB = cardiopulmonary bypass; IGFBP7 = insulin-like growth factor-binding protein; NGAL = neutrophil gelatinase–associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinases-2.

Table 6. Receiver Operating Characteristic Analysis for Persistent Renal Dysfunction at Day 90

	N	N^+	N^-	AUC (95% CI)	*P* Value	N	N^+	N^-	AUC (95% CI)	*P* Value
	Urinary [TIMP-2]·[IGFBP7]					Urinary NGAL				
Preintervention	93	15	78	0.614 (0.470–0.757)	0.121	89	15	74	0.551 (0.411–0.690)	0.478
Postintervention	91	15	76	0.533 (0.373–0.694)	0.684	88	14	74	0.511 (0.368–0.654)	0.879
4-h post CPB	87	15	72	0.692 (0.563–0.821)	0.004	88	15	73	0.657 (0.494–0.819)	0.059
12-h post CPB	92	15	77	0.677 (0.523–0.831)	0.024	85	13	72	0.573 (0.403–0.742)	0.401
24-h post CPB	89	14	75	0.444 (0.268–0.620)	0.535	84	14	70	0.487 (0.311–0.664)	0.887
Composite	94	15	79	0.696 (0.555–0.836)	0.006	89	15	74	0.648 (0.478–0.818)	0.088

AUC = area under the curve; CPB = cardiopulmonary bypass; IGFBP7 = insulin-like growth factor-binding protein; N = all acute kidney injury patients; N^+ = all acute kidney injury patients with positive persistent renal dysfunction at day 90; N^- = all acute kidney injury patients with negative persistent renal dysfunction at day 90; NGAL = neutrophil gelatinase–associated lipocalin; TIMP-2 = tissue inhibitor of metalloproteinases-2.

in critically ill patients after intensive care unit admission similarly correlated with long-term outcomes.[36] However, this subgroup analysis clearly demonstrated that the signal detected by [TIMP-2]·[IGFBP7] is highly specific to AKI.[36] In line with these results, we here demonstrated that [TIMP-2]·[IGFBP7] in the urine immediately after surgery correlates with long-term outcomes but larger randomized controlled trials will have to confirm these findings. However, unlike previous studies, we found that the effects on MAKE at day 90 were not limited to patients manifesting AKI.

Our study has a number of strengths, including the prospective nature of the study together with the scale and completeness of long-term follow-up. Our design allows for greater precision in estimates of absolute risk and increases the clinical applicability of the findings while avoiding potential bias from retrospective selection of the study cohort. In addition, clinical and biochemical outcomes were available for all patients, and data linkage has allowed a complete follow-up of mortality, need for renal replacement therapy, and persistent renal dysfunction. However, a limitation of such a study design is that the findings from a randomized trial are not always generalizable to other patient populations, so caution should be applied in translating these findings from high-risk patients undergoing cardiac surgery to patients undergoing another procedure. Moreover, our measure of kidney dysfunction is limited to serum creatinine at day 90 after surgery. More precise and repeated measures of glomerular filtration rate and possibly biomarkers of kidney damage or assessment of renal reserve may have unmasked further evidence of kidney pathology.

In addition, future studies will need to address the optimal methods for RIPC and whether benefits are consistent across patients with varying risks for AKI defined by either clinical criteria (such as those with preexisting chronic kidney disease or with lower Cleveland Clinical Foundation score) or use of biomarkers. Although we demonstrated that RIPC significantly reduced MAKE, we did not detect a reduction in mortality between the two groups at days 30 and 90 after cardiac surgery. Given the low mortality rates, however, in order to detect a difference in long-term mortality between groups, we would need to analyze more than 10,000 patients. It remains to be determined whether preventing cardiac surgery–associated AKI using RIPC will improve long-term kidney function and outcome, but the effects shown here, especially on dialysis use at 90 days, are encouraging. In conclusion, RIPC significantly improved renal function in high-risk patients undergoing cardiac surgery by reducing prolonged renal dysfunction and need for renal replacement therapy at day 90. Furthermore, the intervention also reduced the 3-month incidence of the composite endpoint MAKE at day 90 without affecting the all-cause mortality. In addition, RIPC not only reduced the severity of AKI but also enhanced renal recovery in those patients who developed AKI.

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Competing Interests

Dr. Zarbock and Dr. Kellum have received grant support and lecture fees from Astute Medical (San Diego, California), unrelated to the current study. Dr. Zarbock and Dr. Kellum have filed a patent application on the use of the biomarkers together with remote ischemic preconditioning. Dr. Meersch has received lecture fees from Astute Medical (San Diego, California), unrelated to the current study. The other authors declare no competing interests.

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Appendix A1: Demographic and Operative Data Further Stratified by Sham-RIPC and RIPC

	$MAKE_{90}^{+}$ (n = 47)			$MAKE_{90}^{-}$ (n = 193)			
	Sham-RIPC (n = 30)	RIPC (n = 17)	Total	Sham-RIPC (n = 90)	RIPC (n = 103)	Total	*P* Value†
Age, yr, mean (SD)	75.5 (5.3)	71.0 (10.4)	73.9 (7.8)	69.0 (10.5)	70.0 (8.9)	69.5 (9.7)	0.002
Male, n (%)	22 (73.3)	6 (35.3)	28 (59.6)	53 (58.9)	70 (68.0)	123 (63.7)	0.597
ASA grade, n (%)							0.321
1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
2	7 (23.3)	4 (23.5)	11 (23.4)	17 (18.9)	23 (22.3)	40 (20.7)	
3	21 (70)	10 (58.8)	31 (66.0)	67 (74.4)	76 (73.8)	143 (74.1)	
4	2 (6.7)	3 (17.7)	5 (10.6)	6 (6.7)	4 (3.9)	10 (5.2)	
CCF score, median (Q1–Q3), points*	6 (6–6)	6 (6–6)	6 (6–6)	6 (6–6)	6 (6–6)	6 (6–6)	0.551
Preoperative creatinine, mean (SD), mg/dl	1.3 (0.5)	1.2 (0.4)	1.3 (0.5)	1.2 (0.4)	1.1 (0.4)	1.1 (0.4)	0.076
eGFR, mean (SD), ml/min per 1.73 m^2	51.3 (11.1)	53.3 (14.8)	52.4 (12.4)	57.9 (16.8)	57.3 (13.2)	57.6 (15.0)	0.027
Creatinine d90, mean (SD), mg/dl	1.8 (0.9)	1.5 (0.5)	1.7 (0.8)	1.1 (0.4)	1.1 (0.3)	1.1 (0.4)	< 0.001
Comorbidities, n (%)							
Hypertension	30 (100)	16 (94.1)	46 (97.9)	86 (95.6)	100 (97.1)	186 (96.4)	1‡
Congestive heart failure	28 (93.3)	17 (100)	45 (95.7)	73 (81.1)	85 (82.5)	158 (81.9)	0.018
Diabetes	11 (36.7)	7 (41.2)	18 (38.3)	33 (36.7)	39 (37.9)	72 (37.3)	0.900
COPD	12 (40)	2 (11.8)	14 (29.8)	28 (31.1)	34 (33.0)	62 (32.1)	0.757
Chronic kidney disease	11 (36.7)	8 (47.1)	19 (40.4)	28 (31.1)	27 (26.2)	55 (28.5)	0.112
Previous heart surgery	3 (10.0)	4 (23.5)	7 (14.9)	11 (12.2)	9 (8.7)	20 (10.4)	0.378
Medication, n (%)							
Aspirin	15 (50)	12 (70.6)	27 (57.4)	51 (56.7)	65 (63.1)	116 (60.1)	0.739
Clopidogrel	4 (13.3)	0 (0)	4 (8.5)	11 (12.2)	11 (10.7)	22 (11.4)	0.568
β blockers	22 (73.3)	12 (70.6)	34 (72.3)	56 (62.2)	56 (54.4)	112 (58.0)	0.072
Statins	23 (76.7)	13(76.5)	36 (76.6)	62 (68.9)	67 (65.1)	129 (66.8)	0.196
Diuretics	21 (70)	9 (52.9)	30 (63.8)	50 (55.6)	54 (52.4)	104 (53.9)	0.218
ACEi or ARBs	20 (66.7)	15 (88.2)	35 (74.5)	53 (58.9)	56 (545.4)	109 (56.5)	0.024
Operative characteristics, n (%)							
Aortic cross-clamp duration, median (Q1–Q3), min	78 (63–113)	91 (62–125.5)	89 (63–120)	78 (57–111)	86 (65–105)	84 (60–110)	0.371
Cardiopulmonary bypass time, median (Q1–Q3), min	127 (89–171)	120 (108–195)	120 (90–175)	113 (90–162)	120 (95–147)	121 (90–178)	0.525
CABG only	9 (30.0)	6 (35.3)	15 (31.9)	27 (30.0)	38 (36.9)	65 (33.7)	0.818
Valve only	2 (6.7)	4 (23.5)	6 (12.8)	19 (21.11)	24 (23.3)	43 (22.3)	0.147
Combined or other	19 (63.3)	7 (41.2)	26 (55.3)	44 (48.9)	41 (39.8)	85 (44.0)	0.164
Postoperative complications, n (%)							
Myocardial infarction	2 (6.9)	1 (6.3)	3 (6.7)	3 (3.3)	5 (4.9)	8 (4.1)	0.468
Major bleeding	8 (27.6)	5 (31.3)	13 (28.9)	10 (11.1)	15 (14.6)	25 (13.0)	0.009
Cerebral insult	0 (0)	1 (6.3)	1 (2.2)	3 (3.3)	1 (1.0)	4 (2.1)	0.950
Cerebral hemorrhage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Pulmonary embolism	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	

*Cleveland Clinic Foundation (CCF) score (0 to 17 points) is composed of 13 preoperative risk factors, comorbidities, and type of surgery. A higher number correlates with a higher rate of dialysis-dependent acute kidney injury after cardiac surgery (Thakar *et al.*[13]). †*P* value for the comparison of the two groups: patients meeting the endpoint major adverse kidney events at day 90 ($MAKE_{90}^{+}$) and patients not meeting the endpoint major adverse kidney events ($MAKE_{90}^{-}$). ‡Fisher exact test.

ACEi = angiotensin-converting enzyme inhibitors; ARB = angiotensin II-receptor blockers; ASA = American Society of Anesthesiology grade (grade 5 patients were not included); CABG = coronary artery bypass graft; COPD = chronic obstructive pulmonary disease; d90 = day 90; eGFR = estimated glomerular filtration rate; RIPC = remote ischemic preconditioning.

Appendix A2: Receiver Operating Characteristic Analysis for Major Adverse Kidney Events at Day 90 (Combination of [TIMP-2]·[IGFBP7] and NGAL)

	Combination [TIMP-2]·[IGFBP7] + NGAL (95% CI)	Δ*P* Value against NGAL	Δ*P* Value against [TIMP-2]·[IGFBP7]
AUC 4 h	0.65 (0.55–0.75)	0.334	0.772
AUC 12 h	0.66 (0.56–0.75)	0.089	0.332
AUC 24 h	0.57 (0.47–0.67)	0.509	0.406
AUC composite	0.65 (0.55–0.74)	0.431	0.501

AUC = area under the receiver operator characteristics curve; IGFBP7 = insulin-like growth factor-binding protein; NGAL = neutrophil gelatinase–associated lipocalin; TIMP-2 = tissue inhibitor of metalloproteinases-2.

Appendix A3: Receiver Operating Characteristic Analysis for Persistent Renal Dysfunction at Day 90 (Combination of [TIMP-2]·[IGFBP7] and NGAL)

	Combination [TIMP-2]·[IGFBP7] + NGAL (95% CI)	Δ*P* Value against NGAL	Δ*P* Value against [TIMP-2]·[IGFBP7]
AUC 4 h	0.66 (0.54–0.78)	0.520	0.519
AUC 12 h	0.60 (0.47–0.73)	0.185	0.978
AUC 24 h	0.54 (0.41–0.66)	0.604	0.577
AUC composite	0.64 (0.52–0.77)	0.717	0.537

AUC = area under the receiver operator characteristics curve; IGFBP7 = insulin-like growth factor-binding protein; NGAL = neutrophil gelatinase–associated lipocalin; TIMP-2 = tissue inhibitor of metalloproteinases-2.