TRIAL PROTOCOL
AND STATISTICAL ANALYSIS PLAN
RENAL EFFECTS OF REMOTE ISCHEMIC PRECONDITIONING IN CARDIAC SURGERY
ACRONYM
RENALRIPC

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Trial protocol code: 05-AnIt-13

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## II. Synopsis

### Principal Coordinating Investigator (PCI)

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### Title of trial

Renal effects of remote ischemic preconditioning in cardiac surgery (RenalRIPC)

### Medical condition

Cardiac surgery with cardiopulmonary bypass

### Objective(s)

Acute kidney injury (AKI) is a well-recognized complication after cardiac surgery with cardiopulmonary bypass (CPB) with an important impact on morbidity and mortality. CPB is employed in most cardiac surgical procedures. Although the mechanisms are not fully understood, ensuing ischemic and inflammatory injuries to renal tubular epithelial cells have been implicated in causing AKI. Despite numerous clinical trials of pharmacologic interventions, a means to prevent AKI associated with cardiac surgery has remained elusive. Remote ischemic preconditioning (RIPC) is a phenomenon in which ischemia–reperfusion (I/R) injury of an organ is mitigated by previous application of brief ischemic episodes in a distant organ or limb. Several encouraging trials of RIPC have suggested clinical benefit. Because the mechanisms of I/R injury are similar to those proposed for AKI after CPB, we will test the hypothesis that RIPC prevents AKI in patients undergoing cardiac surgery.

### Intervention(s)

**Experimental intervention:** RIPC will be induced during general anesthesia prior to cardiopulmonary bypass by three cycles of right upper limb ischemia (high pressure: 5-min blood-pressure cuff inflation to a pressure of 200 mmHg or a pressure that is 50 mm Hg higher than the systolic arterial pressure invasively measured in the radial artery and 5-min cuff deflation; RIPC).  
**Control intervention:** Sham-RIPC intervention will be induced during general anesthesia prior to cardiopulmonary bypass by three cycles of right upper limb ‘pseudo’-ischemia (low pressure: 5-min blood-pressure cuff ‘pseudo’-inflation to a pressure of 20 mm Hg and 5-min cuff deflation) without any limb ischemia (CONTROL).

**Follow-up per patient:** 72 hr postoperatively for laboratory tests and urine output (primary and secondary endpoints); at the day of hospital discharge for laboratory tests; follow up after 30 and 90 days for renal recovery and dialysis-dependency (secondary endpoints).  
**Duration of intervention per patient:** One hour.

### Key inclusion and exclusion criteria

**Key inclusion criteria:** All patients at high risk for AKI, which undergo cardiac surgery with the use of cardiopulmonary bypass. A Cleveland score (Thakar et al. J Am Soc Nephrol. 16: 162-168, 2005) of 6 or more is used to define patients at high risk for AKI.  
**Key exclusion criteria:** Pre-existing AKI, kidney transplantation, chronic kidney disease with a GFR < 30 ml/min, myocardial infarction up to 7 days, age < 18 years, off-pump heart surgery, pregnancy, peripheral vascular disease affecting the upper limbs, hepatorenal syndrome, and drug therapy with sulfonamide and nicorandil.

### Outcome(s)

**Primary efficacy endpoint:** Rate of AKI after cardiac surgery with cardiopulmonary bypass.  
**Key secondary endpoint(s):** Severity of AKI, renal recovery at hospital discharge and after 90 days, 30- and 90-day mortality, need and duration of renal replacement therapy, length of stay on the intensive care unit, total hospital stay, and the determination of different biomarkers.  
Assessment of safety: RIPC has been used in several trials and no side effects have been reported. Perioperative hemodynamics will be monitored. Adverse and serious adverse events in particular those possibly related to RIPC will be documented.

### Trial type

Prospective, multi-centre randomized double-blind, controlled trial.
**Statistical analysis**

Efficacy/test accuracy: The randomized groups will be descriptively compared on all baseline variables using summary statistics such as mean and standard deviation, median and quartiles, or frequency and percent, as appropriate. In inductive statistical analyses two-sided significance tests will be applied with a significance level alpha=0.05, appropriately adjusting for multiple testing. The primary efficacy analysis provides confirmative evidence. Further analyses will be regarded explorative (hypothesis generating) and will be interpreted accordingly. All point estimates of parameters of interest will be supplemented by 95% confidence intervals. SAS or SPSS statistical software will be used for all data analyses.

Description of the primary efficacy/test accuracy analysis and population: The primary efficacy analysis will include all randomized subjects (full analysis set) and will be performed according to the intent-to-treat principle, i.e. all subjects are analyzed in the group to which they were randomized. Additional sensitivity analyses will be performed according to the per-protocol principle. The effect of Sham RIPC versus RIPC on the AKI occurrence will be compared by using a two-sided stratified Chi-Squared test (Cochran–Mantel–Haenszel test, significance level 5%, power 80%).

Safety: Safety data will be evaluated descriptively, including all recruited study patients (safety population). Results are generally reported by mean parameter estimates and associated 95% confidence intervals. Results will be discussed with the Data Monitoring and Safety Board (DMSB).

Secondary endpoints: Statistical analysis of the pre-specified secondary endpoints will be performed with descriptive and inductive statistical methods. Type I error enhancement due to multiple significance testing will be accounted for if applicable.

**Sample size**

To be assessed for eligibility: (n = 2000)
To be assigned to the trial: (n = 240)

**Trial duration**

First patient in to last patient out (months):
Recruitment of patients: 12 months,
Follow up: 12 months
Duration of the entire trial (months): 24 months.

**Participating centres**

To be involved (n=4): Four centres gave signed agreement to participate.
Signed agreement to participate (n=4): University Hospitals of Bochum, Freiburg, Münster, and Tübingen.
## III. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>AKI</td>
<td>Acute Kidney Injury</td>
</tr>
<tr>
<td>AKIN</td>
<td>Acute Kidney Injury Network</td>
</tr>
<tr>
<td>APACHE II</td>
<td>Acute Physiology and Chronic Health Evaluation II</td>
</tr>
<tr>
<td>AT III</td>
<td>Body Mass-Index</td>
</tr>
<tr>
<td>BfArM</td>
<td>Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)</td>
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<tr>
<td>CK</td>
<td>Creatinkinase</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Muscle-Brain type CK</td>
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<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reaktive protein</td>
</tr>
<tr>
<td>DMSB</td>
<td>Data Monitoring Safety Board</td>
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<tr>
<td>eGFR</td>
<td>Estimated glomerula filtration rate</td>
</tr>
<tr>
<td>GDF-15</td>
<td>Growth differentiation factor 15</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HK</td>
<td>Hematokrit</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IGFBP7</td>
<td>Insulin-like growth factor-binding protein 7</td>
</tr>
<tr>
<td>MODS</td>
<td>Multiple Organ Dysfunction Syndrome</td>
</tr>
<tr>
<td>NGAL</td>
<td>Plasma Neutrophil Gelatinase-Associated Lipocalin</td>
</tr>
<tr>
<td>RRT</td>
<td>Renal Replacement Therapy</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SAPS II</td>
<td>Simplified Acute Physiology Score</td>
</tr>
<tr>
<td>SOFA Score</td>
<td>Sequential Organ Failure Assessment Score</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>Tissue inhibitor of metalloproteinases</td>
</tr>
</tbody>
</table>
1. Objective and specific aims

Acute kidney injury (AKI) is a well-recognized complication after cardiac surgery with cardiopulmonary bypass (CPB) with an important impact on morbidity and mortality. CPB is employed in most cardiac surgical procedures. Although the mechanisms are not fully understood, ensuing ischemic and inflammatory injuries to renal tubular epithelial cells have been implicated in causing AKI. Despite numerous clinical trials of pharmacologic interventions, a means to prevent cardiac surgery-associated AKI has remained elusive. Remote ischemic preconditioning (RIPC) is a phenomenon in which ischemia–reperfusion (I/R) injury of an organ is mitigated by previous application of brief ischemic episodes in a distant organ or limb. Several encouraging trials of RIPC have suggested clinical benefit. Because the mechanisms of I/R injury are similar to those proposed for AKI after CPB, we will test the hypothesis that RIPC prevents AKI in patients undergoing cardiac surgery.

Primary efficacy endpoint: The primary endpoint was the occurrence of AKI within the first 72 hrs after surgery. We defined AKI according to the KDIGO criteria.¹

Secondary endpoint(s): Severity of AKI, renal recovery at hospital discharge and after 90 days, 30- and 90-day mortality, need and duration of renal replacement therapy, length of stay on the intensive care unit, total hospital stay, duration of ventilator support and the concentrations of different biomarkers in the first 24 hours after surgery, perioperative myocardial infarction and stroke during the index hospital stay.

Assessment of safety: RIPC has been used in several trials and no side effects have been reported. Perioperative hemodynamics will be monitored. Adverse and serious adverse events in particular those possibly related to RIPC will be documented.

2. Background and significance

2.1. Background

Acute kidney injury (AKI), a significant complication of cardiac surgery, is occurring with greater frequency as patients at high risk for complications are increasingly referred for surgery.²,³ Depending on how it is defined, AKI occurs in up to 45% of patients after cardiac surgery, and approximately 1 to 2% require renal replacement therapy.⁴-⁶ Patients with AKI after cardiac surgery are at risk for lengthened intensive care unit and hospital stays and for short-term and long-term mortality.⁴,⁵ 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 0.3 mg/dl or higher as a diagnostic criterion for AKI.⁷ Cardiopulmonary bypass is employed in most cardiac surgical procedures, and although the mechanisms are not fully understood, ensuing ischemic and inflammatory injuries to renal tubular epithelial cells have been implicated in the cause of AKI.⁸ Despite numerous clinical
trials of pharmacologic interventions, a means to prevent cardiac surgery-associated AKI has remained elusive.8

2.2. Evidence

Ischemic preconditioning is an innate tissue adaptation elicited by ischemia or toxic insult that mediates local and remote organ protection against subsequent exposure to the same or other injury. Local ischemic preconditioning was first observed in dogs as the limitation of myocardial infarct size induced by a series of brief circumflex artery occlusions and reperusions before a more sustained occlusion of the same artery.9 Later studies identified the existence of remote ischemic preconditioning (RIPC), where transient ischemia of many organs and tissues, including heart, liver, and kidney, induced systemic multi-organ protection against subsequent extended ischemia–reperfusion injury.10,11 Ischemic preconditioning is highly conserved across species.10 RIPC suggests the involvement of humoral mediators, and many experiments demonstrate that protection is dialyzable, transferable, and receptor-mediated.10,11 The final common pathway that is triggered by RIPC appears to be a cascade of intracellular kinases with subsequent opening of adenosine triphosphate-sensitive potassium channels and closure of the mitochondrial permeability pore.12 RIPC also induces systemic anti-inflammatory effects and reduces endothelial dysfunction, which may contribute to multi-organ protection.10

It has been known for almost a century that prior injury protects the kidney against subsequent injury,12 with recent studies highlighting modulation of inflammation in ischemic renal preconditioning.13 Nevertheless, most studies of RIPC have focused on the protection of the organs undergoing direct ischemia–reperfusion injury, such as the heart and lungs in cardiopulmonary bypass surgery, and kidneys in aortic surgery.14 However, the systemic multi-organ protection offered by RIPC, including induction of anti-inflammatory and anti-apoptotic gene profiles, suggests potential benefit in prevention of AKI during cardiopulmonary bypass. Indeed, RIPC has shown renal-protective effects in major vascular surgery or interventions without direct interruption of perfusion to the kidneys.14 Nevertheless, renal protection has not been universally observed,15 although problems arise when the assessment of renal dysfunction is an add-on to studies focused on cardiac function.

A number of studies have now addressed renal protection as a primary outcome following RIPC. Firstly, Venugopal et al.16 performed a secondary analysis of renal outcomes of two randomized placebo controlled trials of RIPC for myocardial protection, which used three 5-min cycles of arm ischemia followed by reperfusion in 78 patients undergoing cardiac surgery. This retrospective analysis showed a reduction in the incidence of stages 1, 2, and 3 of AKI by the AKI Network (AKIN) criteria from 25, 0, and 0 % in the control to 3, 8, and 0 % in the RIPC group. Secondly, a prospective randomized double-blind controlled trial of RIPC by Choi et al.17 used three 10-min cycles of lower-limb ischemia and reperfusion in 76 patients undergoing complex valvular cardiac surgery. The primary outcomes were AKI incidence (AKIN definition) and changes in plasma cystatin C at 24 and 48 h after bypass and in the renal injury biomarker plasma neutrophil gelatinase-associated lipocalin (NGAL) at 24 h after bypass. There were no differences in incidence of AKI (12/38 in control and 14/38 in RIPC group) or in the concentrations of renal injury biomarkers between the two groups, although there was a decrease in the creatine kinase MB fraction and in the length of intensive care unit stay in the RIPC group, supporting cardiac benefit. Recently, Zimmerman et al.18 reported a randomized single-blind controlled pilot study of RIPC that used three 5-min cycles of lower-limb ischemia and reperfusion in 120 patients undergoing
cardiopulmonary bypass surgery. The primary outcome was the incidence of AKI (AKIN definition), and the secondary outcomes included change in plasma NGAL 3 h after bypass. There was an absolute risk reduction in AKI of 0.27 (95% confidence interval, 0.10–0.42) with a change in incidence from 28/59 (47%) in the control to 12/59 (20%) in the RIPC group (P = 0.004).

2.3. Significance

Ischemia during cardiac surgery results in a high incidence of AKI that accounts for significant morbidity and mortality. RIPC is a simple, inexpensive procedure with minimal side effects, which can alleviate effects of ischemia. There is considerable evidence from animal studies,19-23 from a number of clinical trials,14,24-26 and from a recent clinical meta-analysis27 that RIPC reduces ischemia-reperfusion injury with respect to biological surrogate endpoints.

However, the elicitation of myocardial protection does not necessarily translate into a readily demonstrable clinical benefit. The currently available studies on the prevention of cardiac surgery-associated AKI by RIPC are underpowered and are not conclusive. Therefore, no specific recommendations can be drawn from these results.

For this reason, a large randomized study is required now with a robust and relevant clinical endpoint. Thus, we project a large multi-centre clinical trial to further scrutinize the effects of RIPC in patients undergoing cardiac surgery focusing on the incidence of AKI.

Given the fact that 1.5 million patients are scheduled for cardiac surgery each year worldwide, a 1/3 risk reduction of the occurrence of AKI by RIPC would result in a remarkable positive effect for many patients, health care systems and health economics.

3. Organisational and administrative aspects of the trial

3.1. Sponsor

Sponsor: Department of Anesthesiology, Intensive Care and Pain Medicine
University Hospital Muenster
Albert-Schweitzer-Campus 1, D5
49149 Muenster
Germany

3.2. Principal Coordinating Investigator

Principal Coordinating Investigator (PCI): Univ. Prof. Dr. A. Zarbock
Department of Anesthesiology, Intensive Care and Pain Medicine
University Hospital Muenster
Albert-Schweitzer-Campus 1, D1
49149 Muenster
Germany
3.3. Statistics

Statistician: Dr. G. Görlich  
Institute of Biostatistics and Clinical Research  
University of Muenster  
Schmeddingstr. 56  
40149 Muenster  
Germany

3.4. Study laboratories and other technical services

Leukocyte Adhesion Laboratory  
Prof. Dr. A. Zarbock  
Department of Anesthesiology, Intensive Care and Pain Medicine  
University Hospital Muenster  
Albert-Schweitzer-Campus 1, A1  
48149 Muenster

3.5. Investigators and trial sites

This clinical trial will be carried out as a multi centre trial in Germany with the University of Münster serving as the Coordinating Center. Sites were chosen based on the following considerations: sample size, timeframe, and projected recruitment rates, willingness and ability of the site to institute all required study interventions successfully and appropriately; and generalizability of findings. The listing of trial sites, principal investigators, subinvestigators, and further trial staff, will be kept and continuously updated in a separate list. The final version of this list will be attached to the final report of the clinical trial.

4. Trial conduct

4.1. General aspects of trial design

The Clinical Trial will be performed as a prospective, randomized, blinded, parallelgroup multicentre trial. Eligible patients will be randomized in a ratio of 1:1 to either Remote ischemic preconditioning (RIPC) or Sham RIPC.

Patients who are considered potential candidates for the study may only participate if signed written informed consent is provided before any study related procedures are initiated (for informed consent procedure see Section 4.3). Each patient for who informed consent is obtained will be assigned a unique patient number. This patient number will be used to identify the patient throughout the study. The patient’s eligibility will be proven by checking the inclusion and exclusion criteria (see Section 4.3).

The randomization number allocates the patient to one of the treatment groups.

4.1.1. Time plan

The study comprises three main periods:

- Period from inclusion and randomization to RIPC or Sham RIPC  
- Observation period (during hospitalization (POD 1, 2, 3 end discharge day)
Follow-up period on day 90 after surgery

End of the clinical trial

The last patient last visit (LPLV) is defined as the end of the clinical trial.

Figure 1 shows the trial work flow. Patients will be identified for recruitment by screening all patients scheduled for cardiopulmonary bypass of participating centers on a daily basis.

4.2. Discussion of trial design

All patients will receive standard perioperative care. RIPC intervention will be performed in group RIPC, and Sham RIPC will be performed in group CONTROL. It has been reported that RIPC is safe and has no side effects.18,26

AKI after cardiac surgery is associated with an increased morbidity and mortality. Despite numerous clinical trials of pharmacologic interventions, a mean to prevent AKI associated with cardiac surgery has remained elusive. In a preliminary study, we demonstrated that the incidence of AKI in the investigated study population (patients at high risk for AKI) is approx. 50% and that there were significantly longer intensive care unit (p=0.001) and hospital stays (p=0.001) in the AKI group.28 Thus, preventing AKI after cardiac surgery would have a great impact on morbidity and mortality.
4.2.1. Randomization

Prior to being randomized into the study, patients will have:

- Signed a written informed consent (see above)
- Completed screening
- Met all designated inclusion/exclusion criteria

Randomization assignment (in a 1:1 ratio to the two treatment arms) will be given only to those patients who fulfilling the inclusion and exclusion criteria and providing informed consent. It will be performed using sealed envelopes. Randomization will be stratified by centre.

4.2.2. Blinding

Study intervention (RIPC or sham RIPC) will be performed by a physician not involved in anesthesia, perioperative care, and endpoint assessment. General anesthesia and intraoperative echocardiography will be performed in all patients by an experienced anesthesia team blinded to the administered inflation pressure of the blood-pressure cuff (high pressure versus low pressure). Standardized perioperative care and management of cardiopulmonary bypass will be provided for all patients blinded to group allocation. Thus blinding concerns i) the individual patient, ii) staff involved in intraoperative (anesthesia and cardiac surgery team) and perioperative care (intensive care unit team), iii) investigators obtaining data, follow-up visits and documentation, and iv) the endpoint committee. Group allocation will not be unfolded until final statistical analysis.

Intention-to-treat analysis will address attrition bias. To prevent publication bias in the future metaanalyses, results are intended to be published irrespective of the outcome of the trial.

4.3. Selection of trial population

4.3.1. Inclusion criteria

1. Patients scheduled for cardiopulmonary bypass
2. Cleveland score of 6 or more
3. Written informed consent

4.3.2. Exclusion criteria

1. Age < 18 years
2. Myocardial infarction up to 7 days before surgery
3. Off-pump heart surgery
4. Preeexisting AKI
5. Kidney transplantation
6. Hepatorenal syndrome
7. chronic kidney disease with a GFR < 30 ml/min
8. peripheral vascular disease affecting the upper limbs
9. Drug therapy with sulphonamide or nicorandil
10. Pregnancy (female patients must be surgically sterile or postmenopausal for at least two years or if of childbearing potential must have a negative serum pregnancy test)

11. Participation in another clinical trial

12. Persons with any kind of dependency on the investigator or employed by the institution responsible or investigator

13. Persons held in an institution by legal or official order

4.4. Withdrawal of trial subjects after trial start

Once a patient has been included in the study the investigator will make every reasonable effort to keep the patient in the study. However, if the investigator has to withdraw a patient from study or if the patient refuses further study participation, a final examination should be performed. For patients withdrawn from the study, the follow-up information should be obtained, if possible.

A patient may request to be withdrawn from the study protocol at any time, for any reason, without prejudice. A patient may also be withdrawn from the protocol at the request of his/her physician, for any reason.

4.4.1. Procedures for premature withdrawal from treatment during the trial

The active study participation stops with the RIPC or Sham RIPC. Patients who withdraw from active study participation will be requested to permit continued data collection for the remainder of the follow-up period.

4.5. Closure of trial sites/Premature termination of the clinical trial

4.5.1. Closure of trial sites or premature termination of trial

The sponsor has the right to terminate the study at a specific study site. Reasons which may require termination are:

- Patient enrolment is too slow
- The investigator fails to comply with the study protocol or legal requirements
- Data recording is not accurate, e.g. CRFs are not completely filled-in or entries are not legible.

4.5.2. Premature termination of trial

The institution responsible has the right to terminate the trial prematurely if there are any relevant medical or ethical concerns, or if completing the trial is no longer practicable. If such action is taken, the reasons for terminating the trial must be documented in detail. All trial subjects still under treatment at the time of termination must undergo a final examination which must be documented. The PCI must be informed without delay if any investigator has ethical concerns about continuation of the trial.

Premature termination of the trial will be considered if:

- The risk-benefit balance for the trial subject changes markedly
• It is no longer ethical to continue treatment
• The institution responsible considers that the trial must be discontinued for safety reasons (e.g. on the advice of the DMC)
• An interim analysis or results of other research show that one of the trial treatments is superior or inferior to another
• It is no longer practicable to complete the trial

The institution responsible decides on whether to discontinue the trial in consultation with the PCI, DMC, Advisory Board and/or statistician.

4.6. Treatment

All patients will receive standard perioperative care with no restrictions in concomitant medication.

4.6.1. Experimental intervention

RIPC will be induced during general anesthesia prior to cardiopulmonary bypass by three cycles of right upper limb ischemia (high pressure: 5-min blood-pressure cuff inflation to 200 mHg or a pressure that is 50 mm Hg higher than the systolic arterial pressure invasively measured in the radial artery and 5-min cuff deflation; RIPC).

4.6.2. Control intervention

Sham-RIPC intervention will be induced during general anesthesia prior to cardiopulmonary bypass by three cycles of right upper limb 'pseudo'-ischemia (low pressure: 5-min blood-pressure cuff ‘pseudo’-inflation to a pressure of 20 mm Hg and 5-min cuff deflation) without any limb ischemia.

4.7. Efficacy and safety variables

4.7.1. Measurement of efficacy and safety variables

4.7.1.1. Primary target variable

The primary endpoint is the occurrence of AKI within the first 72 hrs after surgery. AKI will be defined according to the KDIGO criteria.1

4.7.1.2. Secondary and other target variables

• Severity of AKI within 72 hrs.
• Length of stay in intensive care unit and hospital

Information on ICU and hospital stays will be documented. From admission to hospital respective ICU until follow-up (by phone) at day 90, the location of the patient within the hospital will be documented in the CRF. The following will be recorded for each patient:

- Date and time of admission to hospital respective ICU
- Date and time of discharge from ICU including details of where patient is moving to (e.g. general ward, high dependency unit, etc.)
- Dates, times and primary reason for all admissions to other wards in the hospital and dates and times of discharges from other wards in the hospital
- Dates times and primary reason of all readmissions to ICU and dates and times of discharges from ICU
- Date and time of discharge from hospital
- Dates, times and primary reason of all readmissions to hospital and dates and times of discharges from hospital

- **Need and duration of renal replacement therapy during index hospitalization**

- **Duration of ventilator support**

- **30-day, 90-day mortality**

- **Determination of different biomarkers**

  Urine samples for biomarkers will be collected before RIPC/sham-RIPC, after inducing RIPC or sham-RIPC, and at 4, 12 and 24 h after surgery

- **Incidence of adverse events and serious adverse events (including deaths)**

  All adverse events (AEs) encountered during the clinical study will be reported in detail in the source documents. AEs and SAEs in particular those possibly related to RIPC will be documented. Perioperative complications (myocardial infarction and stroke) will be documented.

4.7.1.3. **Description of visits**

- **Screening, Baseline**
  - Demographic characteristics (date of birth, height, weight, sex)
  - Inclusion and exclusion criteria
  - Result of randomization
  - Anamnesis
  - Laboratory parameter (eGFR, serum creatinine)
  - Blood and urine sampling for determination of biomarkers

- **Surgery day**
  - Respiratory parameter (pH, PaO₂, PaCO₂, SaO₂, FiO₂; ventilation mode, respiratory rate, respiratory minute volume, PEEP)
  - Safety laboratory test
  - Renal parameter (BUN, serum creatinine, urine creatinine, urine volume (ml/hrs) during urine collection, fluid balance)
  - Blood and urine sampling for determination of biomarkers
  - Hemodynamics (MAP, HR, CVP)
  - Concomitant medication
  - Complications
  - Mortality

- **Postoperation day 1**
  - Renal parameter (BUN, serum creatinine, urine creatinine, urine volume (ml/hrs) during urine collection, fluid balance, renal replacement therapy)
  - Blood and urine sampling for determination of biomarkers
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score
  - APACHE II
- SAPS II
- Concomitant medication
- Complications
- Mortality
- Length of Stay

- **Postoperation day 2 and day 3**
  - Renal parameter (BUN, serum creatinine, urine creatinine, urine volume, fluid balance, renal replacement therapy)
  - SOFA-Score
  - APACHE II
  - SAPS II
  - Complications
  - Mortality
  - Length of Stay

- **Discharge day**
  - Safety laboratory test
  - Duration of respiratory support
  - Renal parameter (BUN, serum creatinine, urine creatinine, urine volume, fluid balance, renal replacement therapy, renal recovery)
  - Complications
  - Mortality
  - Length of Stay

- **Day 30**
  - Mortality
  - Length of stay (ICU, Hospital)

- **Day 90**
  - Mortality
  - Length of stay (ICU, Hospital)
Table 1: Investigations during the clinical trial

<table>
<thead>
<tr>
<th>Investigation</th>
<th>T1 Screening / Baseline</th>
<th>T2 (^2) Surgery day</th>
<th>T3 POD 1</th>
<th>T4 POD 2</th>
<th>T5 POD 3</th>
<th>T6 Discharge</th>
<th>T7 Follow-up (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion and Exclusion criteria</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demography (sex, Age, body weight)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Anamnesis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration parameters (pH, P(<em>{O_2}), P(</em>{CO_2}), S(_{O_2}), FiO(_2), ventilation mode, respiratory rate, respiratory minute volume, PEEP)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety laboratory test (sodium, potassium, calcium, chloride, Lactate, Leucocytes, CRP, Procalcitonine, INR, aPTT, Thrombozytes, Fibrinogen, AT III, bilirubine, Hemoglobine, Hematokrit, Erythrozytes, CK, CK-MB)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal parameter (BUN, serum creatinine, urine creatinine, urine volume (ml/h) during urine collection fluid balance)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>x</td>
</tr>
<tr>
<td>Determination of biomarkers</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>(APACHE II, SAPS II, SOFA)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Concomitant medication</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Renal recovery</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mortality</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of stay</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Renal replacement therapy</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Complications</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 The baseline visit will be proceeded normally on day before surgery
2 before RIPC/sham-RIPC, after inducing RIPC or sham-RIPC, and at 4, 12 and 24 h after surgery
3 90 days (80-100 d) after surgery
4.8. Documentation

All data relevant to the trial are documented soon after measurement by the investigator responsible in the case report form supplied. Entering data may be delegated to members of the trial team.

The IT infrastructure and data management staff will be supplied by the Department of Anesthesiology, Intensive Care and Pain Medicine, University Hospital Münster. The trial database will be developed and validated before data entry based on standard operating procedures. The database is integrated into a general IT infrastructure and safety concept with a firewall and backup system. The data are backed up daily. After completion and cleaning of data, the database is locked and the data exported for statistical analysis.

4.8.1. Archiving

All CRFs, informed consent forms and other important trial materials will be archived for at least 10 years.

5. Ethical and regulatory aspects

5.1. Independent ethics committee

The clinical trial will not be started before approval of the competent ethics committee.

In each trial site, the clinical study will not be started before approval of the competent local ethics committee.

The principal investigator will inform the ethics committee about any changes in the study protocol.

5.2. Ethical basis for the clinical trial - Risk/benefit ratio

The present trial protocol and any amendments were and will be prepared in accordance with the Declaration of Helsinki in the version of October 2008 (49th General Assembly of the World Medical Association, Somerset West, Republic of South Africa).

All patients will receive standard perioperative care. No side effects of RIPC have been reported in numerous clinical investigations.\textsuperscript{18,26} None of the patients in both groups (‘RIPC’ and ‘Sham-RIPC’ group) will be exposed to additional risks.

AKI after cardiac surgery is associated with an increased morbidity and mortality. Despite numerous clinical trials of pharmacologic interventions, a means to prevent AKI associated with cardiac surgery has remained elusive. In a preliminary study, we demonstrated that the incidence of AKI in the investigated study population (patients at high risk for AKI) is approx. 50% and that there were significantly longer intensive care unit (p=0.001) and hospital stays (p=0.001) in the AKI group.\textsuperscript{28} Thus, preventing AKI after cardiac surgery would have a great impact on morbidity and mortality.

Participation in this study will be voluntary. Written informed consent will be obtained from patients.

Data collection will be performed pseudonymously and the patient’s name will not appear on any case report form or in any other trial document submitted to the central data management. All collected data will be kept confidential.
The treating investigator will inform the patient about the nature of the trial, its aims, expected advantages as well as possible risks. Each patient must consent in writing to participate in the study. The patient must be given enough time and opportunity to decide on participation and to clarify any questions before the beginning of documentation of the study.

The informed consent will be signed by both patient and treating investigator. The original document is kept by the investigator, whereas the patient receives a copy.

### 5.2.1. Legislation and guidelines used for preparation

The present clinical trial will be conducted in accordance with the published principles of the guidelines for Good Clinical Practice (ICH-GCP). These principles cover, amongst other aspects, ethics committee procedures, the obtaining of informed consent from trial subjects, adherence to the trial protocol, administrative documentation, data collection, trial subjects' medical records (source documents), documentation and reporting of adverse events (AEs), preparation for inspections and audits, and the archiving of trial documentation. All investigators and other staff directly concerned with the study will be informed that domestic and foreign supervisory bodies, the competent federal authorities and authorised representatives of the institution responsible have the right to review trial documentation and the trial subjects’ medical records at any time.

### 5.3. Registration

Before the trial is started, it will be registered under Current Controlled Trials (www.controlled-trials.com) or another trial register approved by the World Health Organisation (WHO) (http://www.who.int/ictrp/en/).

### 5.4. Data protection

The provisions of data protection legislation will be observed. It is assured by the institution responsible that all investigational materials and data will be pseudonymised in accordance with data protection legislation before scientific processing.

Trial subjects will be informed that their pseudonymised data will be passed on in accordance with provisions for documentation and notification pursuant to § 12 and § 13 of the GCP Regulations to the recipients described there. Subjects who do not agree that the information may be passed on in this way will not be enrolled into the trial.

Laboratory data will be maintained in a separate, secure location with access limited only to laboratory personnel.

### 6. Data collection and statistical considerations

#### 6.1. Aim 1 Clinical efficacy

##### 6.1.1. Endpoints

The **primary endpoint** of this trial is the incidence of acute kidney injury (AKI) within 72h after surgery. AKI is defined by the KDIGO criteria.\(^1\) **Secondary endpoints** include the initiation of RRT which will be at the discretion of the ICU clinicians blinded to treatment assignment, the length of ICU stay (days), time on mechanical ventilation (h), myocardial
infarction, the incidence of stroke, length of hospital stay after surgery, all-cause in-hospital mortality and 30-day mortality.

6.1.2. Sample size

Based on our previous study we expected an AKI rate in the control group treated with sham-remote ischemic preconditioning (RIPC) of approx. 50%. The expected absolute risk reduction for AKI is 18% based on a published single-center study investigating the effect of RIPC on AKI after cardiac surgery.18 The primary efficacy analysis is intended to show superiority of RIPC in high-risk cardiac surgery patients, applying a two-sided $\chi^2$ test on significance level $\alpha = 0.05$. The statistical null-hypothesis tests the equality of AKI rates between the two study cohorts. Resulting from these considerations, assuming a power of 80%, 117 evaluable patients per treatment group need to be recruited, i.e. 234 in total. Additional 6 patients will be recruited to account for loss to follow-up or non-evaluable data (drop-out rate ca. 3%). We calculated a necessary sample size based on the primary endpoint using nQuery Advisor software (Version 7).

6.1.3. Analysis plan

The trial is designed to test the primary hypothesis, that RIPC is superior to sham-RIPC (Null-hypotheses: no difference in AKI incidence between RIPC and sham-RIPC). The primary analysis will be based on the intention-to-treat (ITT) collective.

The descriptive analysis of the data will include the calculation of means, standard deviations, and absolute and relative frequencies of the baseline and follow-up data. Randomization will be checked by suitable two-sided statistical tests (Chi-Square, or Fisher’s exact test for categorical data, Students’ t-Test or Mann-Whitney-U tests for continuous data). If normality of the data is not given non-parametric methods will be used.

The primary hypothesis will be answered using a two-sided Chi-Squared test. The primary null hypothesis will be rejected in favor of the alternative hypothesis that the relative frequency of AKI occurrence is different between the two treatment arms, i.e. $p \leq 0.05$.

Potentially confounding factors will be checked for using a multivariable logistic regression analysis. In particular, a full model with clinical relevant covariates (e.g. sex, age, prev. heart surgery, preoperative creatinine, …) will be used for a stepwise backward variable selection procedure to identify independent risk factors for AKI.

Secondary endpoints will be analyzed in the ITT collective using Fishers’ exact test, or chi-squared tests for categorical data, Students’ t-tests and Mann-Whitney-U tests for continuous data.

6.2. Aim 2: Biological mechanisms of action

Primary objective of Aim 2 are to the hypothesis that RIPC protects the kidney from an injury caused by cardiopulmonary bypass during cardiac surgery. RIPC releases different mediators (high mobility group box 1, HMBG-1) that are filtered in the glomerulus and induces a cell cycle arrest (insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2)) in tubular epithelial cells.
6.2.1. Approach for Aim 2

We will measure biomarkers in the urine at different time points during the study, i.e. before RIPC, immediately after RIPC, 4h, 12h, and 24h after cardiopulmonary bypass. [TIMP-2]•[IGFBP7] will be measured by point-of-care diagnostics, HMBG-1 and neutrophil gelatinase-associated lipocalin (NGAL) will be determined by using commercially available assays. Analyses will be performed centrally at the university hospital Münster, Germany by blinded lab technicians.

6.2.2. Analysis plan for Aim 2

The biomarker levels at each time point will be described by mean and standard error.

The two study arm RIPC and sham-RIPC will be compared for differences in biomarker concentration using Mann-Whitney-U tests, at each individual time point.

Additionally, we will fit a multivariable logistic regression model to identify the association of covariates with increased in [TIMP-2]•[IGFBP7] level (threshold 0.5).

An association of (discretized) [TIMP-2]•[IGFBP7] levels with AKI occurrence will be tested by chi-squared tests.

7. Use of trial findings and publication

7.1. Publication

It is planned to publish the trial results, in mutual agreement with the PCI, in a scientific journal and at German or international congresses. Publication of the results of the trial as a whole is intended. Any publication will take account of the ‘(International Committee of Medical Journal Editors’ (ICMJE)).

The trial will also be registered in a public register in accordance with the recommendations of the ICMJE (see also Section 5.3).

Any published data will observe data protection legislation covering the trial subject and investigators. Success rates or individual findings at individual trial sites are known only to the institution responsible.

Publications or lectures on the findings of the present clinical trial either as a whole or at individual investigation sites must be approved by the institution responsible in advance, and the institution responsible reserves the right to review and comment on such documentation before publication.

8. Costs and payments

8.1. Research study costs

There will be no additional costs to subjects as part of this study. The only additional study costs above what is considered to be standard hospital care are the costs of the measurement of the biomarkers. These costs will be covered by the study coordination
center in Münster and a research grant of the German Research Foundation. Subjects and their insurers or third party payers will not be billed for research related services. All research related services (central laboratory supplies/services) will be paid for by the study sites. Subjects and their insurers and third party payers will be billed for routine care services, or services not connected with the study. These routine care services include services provided during this hospitalization and any ongoing services or medications required after leaving the hospital. Subjects will be responsible for any applicable copays, coinsurances, and deductibles.

8.2. Research study payments

Research subjects will receive no payments or other remuneration for their participation in the study.

9. Amendments to the trial protocol

To ensure that comparable conditions are achieved as far as possible at individual trial sites and in the interests of a consistent and valid data analysis, changes to the provisions of this trial protocol are not planned. In exceptional cases, however, changes may be made to the trial protocol. Such changes can only be made if agreed by the institution responsible, the PCI and biometrician, and all Authors of this trial protocol. Any changes to the trial procedures must be made in writing and must be documented with reasons and signed by all Authors of the original trial protocol.

Amendments made in accordance with § 10 Secs. 1 and 4 GCP Regulations that require approval are submitted to the ethics committee and the supreme federal authority and will not be implemented until approved. Exceptions to this are amendments made to avoid immediate dangers.

10. References


