TRIAL PROTOCOL
AND STATISTICAL ANALYSIS PLAN
BIOMARKER GUIDED IMPLEMENTATION OF THE KDIGO GUIDELINES TO REDUCE THE OCCURRENCE OF AKI IN PATIENTS AFTER CARDIAC SURGERY
ACRONYM
PREVAKI

Institution Responsible:	Principal Coordinating Investigator:
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Trial protocol code: 01-AnIt-14

Version of 03.04.2014, V1.

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I.   Synopsis

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Title of the trial	Biomarker-guided implementation of the KDIGO guidelines to reduce the occurrence of acute kidney injury in patients after cardiac surgery
Short Title	PrevAKI
Study-ID	01-AnIt-14
Responsible institution	Department of Anesthesiology, Intensive Care and Pain Medicine Albert-Schweitzer-Campus 1, A1 48149 Muenster
Medical condition	Elective cardiac surgery with extracorporeal circulation
Trial type	Single-center, prospective, randomized controlled parallel group clinical trial
Objective(s)	Acute kidney injury (AKI) is a well-recognized complication after cardiac surgery with cardiopulmonary bypass (CPB) with an important impact on short- and long-term morbidity and mortality. CPB is employed in most cardiac surgical procedures. Despite numerous clinical trials testing different pharmacological treatments, a mean to prevent cardiac surgery-associated AKI (CSA-AKI) remains elusive. In 2012 the Kidney Disease: Improving Global Outcomes (KDIGO) workgroup published the KDIGO guidelines which recommend the implementation of a bundle of supportive measures to prevent AKI in high risk patients (discontinue all nephrotoxic agents when possible, discontinue angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in the perioperative period to avoid severe hypotension, optimizing volume status and hemodynamic parameters, consider functional hemodynamic monitoring, monitor serum creatinine and urine output, avoid hyperglycemia, consider alternatives to radiocontrast procedures). However, there is no evidence whether this bundle of supportive measures actually prevent AKI.
Intervention	Patients at high risk for the development of AKI will be identified by measuring [TIMP-2]*[IGFBP7] (NephroCheck® Test) 4h after cardiopulmonary bypass. Patients with NC ≥ 0.3 will be randomized. Validation group: Implementation of the KDIGO guidelines: Optimizing volume status and hemodynamic parameters (according to a hemodynamic algorithm, see page 17), discontinuation of all nephrotoxic agents when possible, discontinue angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in the perioperative period to avoid severe hypotension, consideration of alternatives to radiocontrast agents, close monitoring of serum creatinine and urinary output, avoidance of hyperglycemia. Control group: Standard of care: mean arterial pressure (MAP) > 65 mmHg, central venous pressure (CVP) 8-10 mmHg
Key inclusion and exclusion criteria	Inclusion criteria: 1. Adult patients undergoing cardiac surgery with CPB 2. [TIMP-2]*[IGFBP7] ≥ 0.3 4h after CPB 3. Age between 18-90 years 4. Written informed consent Exclusion criteria: 1. Preexisting AKI 2. Pregnancy 3. (Glomerulo-) Nephritis, interstitial nephritis or vasculitis 4. CKD with eGFR < 30 ml/min 5. Dialysis dependent CKD 6. Prior kidney transplant 7. Patients on mechanical assist devices (ECMO, LVAD, RVAD, IABP) 8. Participation in another clinical intervention trial

	9. Persons with any kind of dependency on the investigator or employed by the institution responsible or investigator 10. Persons held in an institution by legal or official order
Primary trial objective	To investigate whether the bundle of supportive measures recommended in the KDIGO guidelines reduces the occurrence of CSA-AKI in high risk patients.
Study endpoints	Primary endpoint: • Occurrence of AKI according to the KDIGO guidelines within 72 hours after cardiac surgery Secondary endpoints: • Severity of AKI (according to the KDIGO guidelines) • Renal recovery at days 30, 60 and 90 • 30-day, 60-day and 90-day mortality • Length of ICU stay • Length of hospital stay • Need and duration of renal replacement therapy • RRT at days 30, 60, 90 • $MAKE_{30}$, $MAKE_{60}$ and $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency persistent renal dysfunction (defined as serum creatinine ≥ 0.5mg/dl compared to baseline value) • Determination of different biomarkers • Assessment of safety: adverse and serious adverse events in particular those related to the implementation of the KDIGO guidelines will be documented. No side effects have been reported.
Number of subjects	To be assigned to the trial: n=276 (138/ treatment arm)
Time plan	First patient first visit (FPFV): 1 July 2014 Last patients first visit (LPFV): 30 September 2015 Last patient last visit (LPLV): 31 December 2015 Final study report: 30 June 2016
Statistics	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 validation versus control care 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. 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.
Trial Registration	DRKS 00006139

I. Abbreviations

Abbreviation	Meaning
ACE	Angiotensin converting enzyme inhibitor
AE	Adverse event
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
ALI	Acute lung injury
APACHE II	Acute Physiology and Chronic Health Evaluation II
ARB	Angiotensin receptor blocker
ARDS	Acute respiratory distress syndrome
BMI	Body Mass Index
BUN	Blood urea nitrogen
CK	Creatine kinase
CKD	Chronic kidney disease
CK-MB	Muscle-Brain type CK
CPB	Cardiopulmonary bypass
CRF	Case report form
CRP	C-reactive protein
CSA-AKI	Cardiac surgery-associated acute kidney injury
CVP	Central venous pressure
ECMO	Extracorporeal membrane oxygenation
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
Hb	Hemoglobin
Hk	Hematokrit
IABP	Intra-aortic balloon pump
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IGFBP7	Insulin-like growth factor-binding protein 7
KDIGO	Kidney Disease: Improving Global Outcomes
LVAD	Left ventricular assist device
MAKE	Major adverse kidney event
MAP	Mean arterial pressure
PCI	Principal coordinating investigator
PTT	Partial thomboplastin time
RIFLE	Risk, Injury, Failure, Loss classification
RRT	Renal replacement therapy
RVAD	Right ventricular assist device
SAPS II	Simplified Acute Physiology Score
SOFA Score	Sequential Organ Failure Assessment Score
TIMP-2	Tissue inhibitor of metalloproteinases

1 Objective and specific aims

Acute kidney injury (AKI) is a well-recognized complication after cardiac surgery with an important impact on short- and long-term morbidity and mortality. Cardiopulmonary bypass (CPB) is employed in most cardiac surgical procedures. Despite numerous clinical trials testing different pharmacological treatments, a mean to prevent cardiac-surgery associated AKI (CSA-AKI) remains elusive. In 2012 the Kidney Disease: Improving Global Outcomes (KDIGO) workgroup published the KDIGO guidelines which recommend to implement a bundle of supportive measures in patients at high risk for AKI to prevent the development of AKI (discontinuation of all nephrotoxic agents when possible, optimizing volume status and hemodynamic parameters, discontinue angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in the perioperative period to avoid severe hypotension, consider functional hemodynamic monitoring, monitor serum creatinine and urine output, avoid hyperglycemia, consider alternatives to radio contrast procedures). However, there is no evidence whether this bundle of supportive measures actually prevents AKI.

Primary efficacy endpoint:	The primary endpoint is the occurrence of AKI within the first 72 hours after cardiac surgery. We define AKI according to the KDIGO guidelines (1).
Secondary endpoint(s):	Severity of AKI, renal recovery at days 30,60 and 90, 30-day, 60-day and 90-day mortality, length of ICU stay, length of hospital stay, need and duration of RRT, RRT at days 30, 60 and 90, $MAKE_{30}$, $MAKE_{60}$ and $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency persistent renal dysfunction (defined as serum creatinine ≥ 0.5mg/dl compared to baseline value).
Assessment of safety:	No side effects are expected by implementing the KDIGO guidelines.

2 Background and significance

2.1 Background

AKI is defined as an acute loss of renal function developing over a period of hours to days and represents a common complication in patients undergoing cardiac surgery (2) . 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 (RRT) (3-5). It may occur in patients with preoperative normal kidney function as well as in patients with chronic kidney disease (CKD) (6). Several

clinical conditions e.g. sepsis, hypovolemia, extended surgical procedures (cardiac surgery with CPB) and the use of nephrotoxic agents can result in the development of AKI. Complications associated with AKI, such as electrolyte imbalances, fluid overload and uremia can lead to life-threatening conditions and worsen patients' outcome.

CPB 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 (7). Despite numerous clinical trials of pharmacological treatments, a mean to prevent CSA-AKI has remained elusive.

2.2 Evidence

Independent of the underlying disease, AKI is associated with an increased morbidity and mortality, especially in patients undergoing cardiac surgery with CPB (8, 9). Numerous studies demonstrate that 1/3 of patients undergoing cardiac surgery suffer from AKI in the postoperative period (7). Although most of the patients develop only a mild AKI, mortality rates are 5 times higher compared to patients without the development of AKI in the postoperative period (10). Minimal elevations of serum creatinine of 0.3 mg/dl are associated with higher mortality rates. Furthermore, patients surviving severe AKI are at high risk for the development of CKD and end-stage renal disease (ESRD) leading to worse long term outcome and a tremendous economic burden for the health care system. These facts give treatment strategies for the prevention of AKI a high priority (11).

In 2012, the KDIGO group worked out some strategies for the prevention of AKI (12). These include the discontinuation of all nephrotoxic agents when possible, consideration of alternatives to radiocontrast agents, optimizing volume status and hemodynamic parameters, discontinue angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in the perioperative period to avoid severe hypotension, the consideration of a functional hemodynamic monitoring, close monitoring of serum creatinine and urinary output and the avoidance of hyperglycemia. Whether this bundle of supportive measures is effective to prevent AKI needs to be proven.

In the perioperative period several classes of nephrotoxins are used, including antibiotics (e.g vancomycin (13) and aminoglycosides (14)) and intravenous contrast agents (15). A number of studies have shown that these nephrotoxic agents may lead to a deterioration of renal function and consequently to AKI (13-16).

ACE-inhibitors and ARBs are used to treat hypertension. A lot of patients undergoing cardiac surgery are on ACE inhibitors or ARBs which may cause a severe and prolonged hypotension during anesthesia. These hypotensive episodes may lead to a reduced renal perfusion. Walsh et al. performed a cohort study on 33.330 patients undergoing non-cardiac surgery and

demonstrated that intraoperative hypotensive episodes of less than 5 minutes with an MAP < 55 mmHg were associated with a significantly increased AKI rate (17). These data support the recommendation why the discontinuation of ACE inhibitors and ARBs in the perioperative period and a functional hemodynamic monitoring might be helpful to prevent the development of AKI.

Close monitoring of the classic biomarkers serum-creatinine and urinary output are important to early detect a functional damage. The avoidance of hyperglycemia results from the landmark study performed by Van den Berghe et al. who showed a significant reduction of AKI in patients receiving strict glucose control (18). On the contrary, there are several trials showing that strict glucose control might not only be associated with higher AKI incidence but also with higher mortality rates (19, 20).

The severity of AKI is classified into different stages by various classification systems (RIFLE, AKIN, KDIGO) based on reduction of urinary output and/or an increase of serum creatinine compared to baseline (1, 21). Both „classic biomarkers" are not appropriate for the detection of kidney damage without a loss of function, because both markers are functional markers. Urinary output is influenced by the use of diuretics and fluid status (e.g. hypervolemia) and has a low specificity. Serum creatinine elevations are not detectable until 50% of the glomerular filtration rate (GFR) is lost, showing that this parameter has a low sensitivity. However, the injury occurs at a much earlier time point. New biomarkers such as [TIMP-2]*[IGFBP7] are injury markers demonstrating an injury of the kidneys without a loss of kidney function (22). We performed an observational trial demonstrating that urinary [TIMP-2]*[IGFBP7] levels 4h after cardiac surgery showed a good performance to predict AKI within the next 72h (NC 0.3: sensitivity 0.80, specificity 0.83, PPV 0.80; NPV 0.83) (23). In those patients with AKI, biomarker elevations were apparent two days before the increase of serum-creatinine.

In this current trial we will include cardiac surgery patients who are at increased risk for AKI (defined as TIMP-2*IGFBP7 ≥ 0.3) and analyze whether the implementation of the bundle of supportive measures recommended by the KDIGO guidelines helps preventing the development of CSA-AKI.

2.3 Rationale

CPB is frequently used in cardiac surgery and the use leads to ischemic, inflammatory and oxidative damages of renal tubular epithelial cells. However, the mechanism of renal tubular epithelial cell damage is still unknown. Although numerous studies have investigated different pharmacological interventions to prevent AKI, effective strategies are missing. The KDIGO guidelines for AKI recommend some non-interventional strategies for the prevention of AKI:

- Discontinuation of all nephrotoxic agents when possible

- Optimization of volume status and hemodynamic parameters including perfusion pressure

- Consideration of functional hemodynamic monitoring

- Close monitoring of serum creatinine and urine output

- Avoidance of hyperglycemia

- Consideration of alternatives to radio contrast agents

- Discontinue ACE-inhibitors and ARBs

Although the evidence for the different supportive measures is really weak, the KDIGO guidelines recommend to implement them in patients at high risk for AKI. Therefore, a randomized prospective trial is needed to investigate whether the implementation of the bundle of measures is effective to prevent AKI in high risk patients undergoing cardiac surgery.

2.4 Benefit-risk assessment

Patients included in the trial have an increased risk for the development of AKI.

In the validation group, the bundle of supportive measures recommended by the KDIGO guidelines will be implemented, whereas patients in the control group receive standard of care therapy (MAP > 65 mmHg and CVP between 8 and 10 mmHg). The intervention may reduce the occurrence and severity of AKI.

The KDIGO guidelines include optimization of volume status and hemodynamic parameters, discontinuation of nephrotoxic agents when possible, discontinuation of ACE-inhibitors and ARBs, close monitoring of serum creatinine and urinary output, and avoidance of hyperglycemia. To this date, the benefit of these simple and uncomplicated measures has not been proven. Patients assigned to the control group will not have any disadvantages compared to non-participation in the study.

All the patients included in this trial receive standard therapy according to the current clinical routine. The patient's primary physicians will determine the remainder of patient management consistent with established best practices with the management of cardiac surgery patients.

2.5 Significance

The occurrence of AKI after cardiac surgery is associated with an increased morbidity and mortality. The KDIGO guidelines recommend to implement some measures in high risk patients to prevent AKI. However, it has not been proven whether the implementation of these measures reduces the occurrence of AKI after cardiac surgery. Patients at high risk for AKI will be identified by using the AKI biomarkers [TIMP-2]*[IGFBP7] which will be measured 4h after cardiac surgery.

Given the fact that 1.5 million patients are scheduled for cardiac surgery each year worldwide, reducing the occurrence of AKI by biomarker-guided implementation of the KDIGO guidelines

would result in a remarkable positive effect for many patients, health care systems and health economics.

3 Organizational 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, Geb. A1
                   48149 Muenster

                   Germany

3.2 Principal Coordinating Investigator

Principal Coordinating

Investigator (PCI):   **Univ. Prof. Dr. med. A. Zarbock**

                   Department of Anesthesiology, Intensive Care and Pain Medicine
                   University Hospital Muenster
                   Albert-Schweitzer-Campus 1, Geb. A1
                   48149 Muenster
                   Germany

3.3 Statistics

Statistician: Dr. rer. nat. J. Gerß
                   Institute of Biostatistics and Clinical Research
                   University of Muenster
                   Schmeddingstr. 56

                   48149 Muenster
                   Germany

3.4 Study laboratories and other technical services

Leukocyte Adhesion Laboratory

Prof. Dr. med. 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 single-center trial at the University Hospital of Muenster.

4 Trial conduct

4.1 General aspects of trial design

The Clinical Trial will be performed as a prospective, randomized, blinded, parallel group single center trial. Eligible patients will be randomized in a ratio of 1:1 to receive either interventional or standard treatment.

Patients who are considered potential candidates for the study may only participate if signed written informed consent is provided or the specific process for unconscious patients in an emergency situation is followed before any study related procedures are initiated (see 4.3.1). 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.

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 48 h (end of the intervention period)
- Observation period (during hospitalization)
- Follow-up period on day 90 after patient enrolment.

End of the clinical trial

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

4.2 Discussion of trial design

All patients will receive standard perioperative care. In the validation group, patients will be strictly treated according to the KDIGO guidelines and following a special algorithm to optimize volume status and hemodynamic parameters. The KDIGO recommendations are safe and have no side effects. In the control group, patients will receive standard care.

AKI after cardiac surgery is associated with an increased morbidity and mortality. Numerous clinical trials tested different pharmacological treatments, however no agents have been demonstrated to be efficacious in clinical practice. In a preliminary study, we demonstrated that urinary [TIMP-2]*[IGFBP7] levels 4 hours after CPB showed good performance in predicting the development of AKI (AUC 0.81). Moreover, patients with a cut-off value ≥ 0.3 $((ng/ml)^2/1000)$ were identifiedas patients with a high risk for AKI (5-times higher than [TIMP-2]*[IGFBP7] < 0.3; p< 0.001). 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
- 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 fulfilling the inclusion and none of the exclusion criteria and providing informed consent. Randomization codes will be computer generated and concealed from investigators. After identifying a high risk patient (urinary [TIMP2]*[IGFBP7] ≥ 0.3, NephroCheck® Test), patients will be assigned to control or intervention group, whereas the intervention will be provided by an investigator not involved in the care of patient. No stratification will be performed.

4.2.2 Blinding

Study intervention will be performed by a physician **not involved** in anesthesia, perioperative care, and endpoint assessment. General anesthesia and perioperative care will be performed in all patients by an experienced anesthesia team. Thus, blinding concerns i) the individual patient, ii) investigators obtaining data, follow-up visits and documentation, and iii) 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 future meta-analyses, results are intended to be published irrespective of the outcome of the trial.

4.3 Selection of trial population

4.3.1 Consent and recruitment strategy

All adult patients fulfilling the inclusion criteria will be offered enrollment in the study. However, patients who enter the ICU after cardiac surgery are mostly sedated and mechanically ventilated. Biomarker elevations detect a potential renal damage at an early time point and interventions have to begin in this early phase to prevent the development of AKI. This practically emergency condition does not allow delaying the intervention until patients' consciousness. For this

unconscious emergency situation, the informed consent process has to follow the legal country-specific regulations. On the basis of the German Civil Code (§ 1902 and § 1904) and on the basis of the German Drug Law (§40 and § 41) the following informed consent process is defined for Germany.

A legally authorized representative may provide the written informed consent in case of an emergency situation where the patient is not capable of signing informed consent. If no legally authorized representative is available or no legally authorized representative is appointed by the local court, this authorization has to be initiated. If the patients' treatment in an emergency situation may not allow any delay and if the legally authorized representative cannot be appointed in a timely manner, a declaration about the patients' inclusion and about the patients' unconsciousness has to be obtained from an experienced consultant physician who is not involved in the study and who is independent of the investigational team. This procedure has to be documented on the declaration form.

It is strongly recommended to ask as soon as possible a relative or an associated person about the patient´s presumed will and any previous statement of the patient not to be willing to participate in clinical studies. The information has to be documented in the patient's medical record. Once the patient regains the capability of providing informed consent he/ she needs to be asked for his/her informed consent to continue with the study and those forms need to be filled in the study documents.

The patient needs to be informed that all the data are kept confidential and in pseudonymized form. He/ she has the right to withdraw study participation at any time point of the study without giving any reason. A duplicate of the written informed consent needs to be handed out.
No patient will be excluded from the study on the basis of gender, race, ethnicity or sexual preference. Patients will be identified for recruitment by screening patients receiving care in the critical care units.

4.3.2 Inclusion criteria

1. Patients undergoing cardiac surgery with cardiopulmonary bypass (CPB)

2. Urinary [TIMP-2]*[IGFBP7] ≥ 0.3 4 h after CPB

3. Age between 18 and 90 years

4. Written informed consent

4.3.3 Exclusion criteria

None of the following conditions can be fulfilled:

1. Preexisting AKI

2. Pregnancy

3. (Glomerulo-) Nephritis, interstitial nephritis or vasculitis

4. CKD with eGFR < 30 ml/min

5. Dialysis dependent CKD

6. Prior kidney transplant

7. Patients on mechanical assist devices (ECMO, LVAD, RVAD, IABP)

8. Participation in another clinical interventional trial

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

10. 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 the 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 end of the intervention period (48h after randomization). 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 site/ premature termination of the clinical trial

4.5.1 Closure of trial site

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 the clinical trial

The responsible institution (University Hospital Muenster) 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 responsible institution decides on whether to discontinue the trial in consultation with the PCI, Advisory Board and/or statistician.

4.6 Treatment

All patients will receive standard of care therapy. In the validation group, optimization of fluid status and hemodynamic parameters according to a specific algorithm (see Figure 1), avoidance of hyperglycemia, discontinuation of nephrotoxic drugs if possible, consideration of alternatives to radio contrast agents, and the discontinuation of ACE inhibitors and ARBs, and close monitoring of serum creatinine and urine output will be performed. Further concomitant medication will not be restricted.

Figure 1. Hemodynamic algorithm (validation arm)
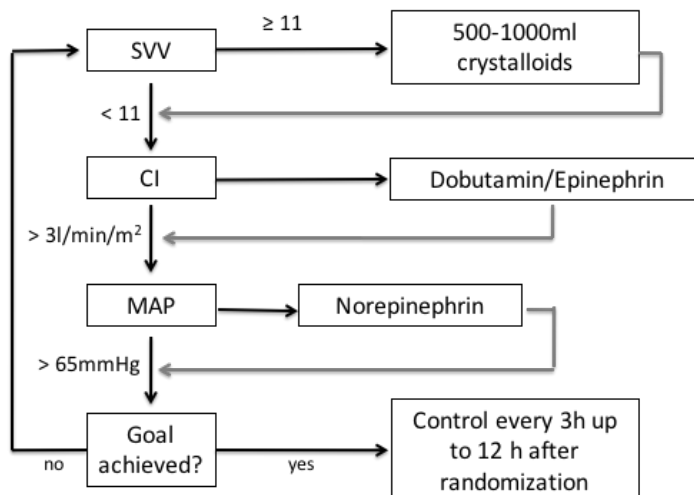
4.6.1 Experimental intervention

Four hours after terminating CPB in the context of a cardiac surgery procedure, urinary [TIMP-2]*[IGFBP7] will be measured to identify patients at high risk for AKI. Patients with a value ≥ 0.3 will be randomized as follows to one of the two treatment arms.

The KDIGO guidelines for prevention of acute kidney injury will be strictly implemented:

- Discontinuation of all nephrotoxic agents if possible

- Discontinuation of ACE inhibitors and ARBs for the first 2 days after surgery

- Close monitoring of serum creatinine and urinary output

- Avoidance of hyperglycemia by close monitoring

- Consideration of alternatives to radio contrast agents

- Hemodynamic monitoring and optimization according to a hemodyamic algorithm (see Fig. 1)

4.6.2 Control intervention

Patients in the control group will receive standard of care. MAP will be kept > 65 mmHg and CVP between 8 and 10 mmHg.

4.6.3 Additional treatments

The patient's primary physicians will determine the remainder of patient management consistent with established best practices with the management of critically ill patients. In patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), tidal volume for mechanical ventilation will be approximately 6 ml per kilogram of predicted body weight and adjusted to maintain a peak plateau pressure between 25 and 30 cm of water (24). Ventilator associated pneumonia will be evaluated and treated in accordance with published clinical practice guidelines and consensus statements (25). Sepsis will be diagnosed and treated in accordance with published guidelines (26). All medications will be dose adjusted for renal failure and RRT in accordance with standard dosing guidelines.

All patients will be prescribed a nutritional intake that will provide at least 25-30 kcal/kg/day, depending on mechanical ventilation and other factors. Protein intake will be at least 1.2 g/kg/day. In patients receiving parenteral nutrition, carbohydrate infusion rates will not exceed 5 mg/kg/min. Water-soluble vitamins will be supplemented to replace dialysis-related losses.

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 hours 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
- Renal recovery

  Renal recovery will be defined as serum-creatinine at hospital discharge and at days 30, 60 and 90 ≥ 0.5 mg/dl compared to baseline value. Additionally, we will include a second definition for renal recovery (serum-creatinine at hospital discharge and at days 30, 60 and 90 ≥ 2-times higher compared to baseline value).

- 30-day, 60.day and 90-day mortality
- 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 RRT
- RRT at days 30, 60 and 90
- $MAKE_{30}$, $MAKE_{60}$ and $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency persistent renal dysfunction (defined as serum creatinine ≥ 0.5mg/dl compared to baseline value)
- Determination of different biomarkers

Urine samples for biomarkers will be collected at the time of randomization and 12 hrs later

- 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 Visits in detail

	T1 Base-line	T2[1] OD	T3 POD 1	T4 POD 2	T5 POD 3	T6 Dis-charge	T7 Follow-up[2]
Inclusion and exclusion criteria	X						
Randomization	X						
Demographic Data (age, gender, BMI)	X						
Medical history	X						
Respiratory parameter (pH, P_aO_2, P_aCO_2, S_aO_2, FiO_2, ventilation mode, P_{insp}, PEEP)	X	X					
Laboratory parameter (sodium, potassium, chloride, lactate, leucocytes, CRP, PCT, Quick, PTT, thrombocytes, bilirubin, hemoglobin, CK, CK-MB)	X	X				X	
Renal parameter (BUN, creatinine, GFR, urine volume [ml/h], urinary output, fluid balance)	X	X	X	X	X	X	X
Biomarker	X	X					
Scores (APACHE II, SAPS II, SOFA)		X	X	X	X		

[1] After randomization
[2] d30, d60, d90

Concomitant medication (vasopressors, radio contrast agents, diuretics)		X	X				
Renal recovery		X	X	X	X	X	X
Mortality							X
Length of ICU and hospital stay							X
Renal replacement therapy		X	X	X	X	X	X
MAKE							X
Complications (myocardial infarction, cerebral ischemia, ICB, bleeding >300ml, re-operation)			X	X	X	X	X

4.8 Documentation

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

The information technology (IT) infrastructure and data management staff will be supplied by the Department of Anesthesiology, Intensive Care and Pain Medicine, University Hospital Muenster. The trial database will be developed and validated before data entry based on standard operating procedures (SOPs). 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.

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 the implementation of the KDIGO guidelines are known. None of the patients in both groups 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 urinary [TIMP-2]*[IGFBP7] levels 4 hours after CPB showed a good performance in predicting the development of AKI (AUC 0.81). Moreover, patients with a cut-off value ≥ 0.3 $((ng/ml)^2/1000)$ were identified as patients with a high risk for AKI (5-times higher than [TIMP-2]*[IGFBP7] < 0.3; p< 0.001). 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. If the patient is unable to provide informed consent, the legally authorized representative has to provide the written informed consent or in her/his absence a declaration for inclusion in an emergency situation is to be signed by a consultant physician who is not involved in the study and who is independent of the investigational team. Patient or legally authorized representative informed consent will be obtained as soon as the patient's condition allows it.

Data collection will be performed pseudonymously and the patient's name will not appear on any CRF 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 or the legally authorized representative in case of patients' unconsciousness 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 or legally authorized representative in case of patients' unconsciousness 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/ legally authorized representative 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 responsible institution 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 responsible institution that all investigational materials and data will be pseudonymised in accordance with the 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 Statistics

6.1 Endpoints

The primary endpoint of this trial is the occurrence of acute kidney injury (AKI) within 72h after cardiac surgery. AKI is defined by the KDIGO criteria (1).
Secondary endpoints include severity of AKI, renal recovery at days 30,60 and 90, 30-day, 60-day and 90-day mortality, length of ICU stay, length of hospital stay, need and duration of RRT, RRT at days 30, 60 and 90, $MAKE_{30}$, $MAKE_{60}$ and $MAKE_{90}$ (major adverse kidney events consisting of mortality, dialysis dependency persistent renal dysfunction (defined as serum creatinine ≥ 0.5mg/dl compared to baseline value).

The randomization ratio is 1:1. No stratification is planned.

6.2   Sample size

Based on previous trials the incidence of those patients with [TIMP-2]*[IGFBP7] ≥ 0.3 is 80% (sensitivity 0.8, specificity 0.83, PPV 0.8, NPV 0.83). We hypothesize to reduce the occurrence of AKI to 65% (expected absolute risk reduction for AKI is 15%, there are no reference values due to the lack of published trials). The primary endpoint will be analyzed using the chi square test (5% level). The primary efficacy analysis is intended to show superiority of the intervention in high-risk cardiac surgery patients, applying a two-sided χ^2 significance level α = 0.05. The statistical null-hypothesis tests the equality of AKI rates test in between the two study cohorts. Resulting from these considerations, assuming a power of 80%, 138 evaluable patients per treatment group need to be recruited, i.e. 276 in total. We calculated a necessary sample size based on the primary endpoint using nQuery Advisor software (Version 7).

6.3   Statistical analysis plan

The trial is designed to test the primary hypothesis, that the intervention is superior to non-intervention (Null-hypotheses: no difference in AKI incidence between intervention and control). 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, previous 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.

7 Use of trial findings

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.

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 responsible institution.

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 responsible institution in advance, and the responsible institution 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 and for a study nurse. These costs will be covered by the center in Muenster, a research grant of the IMF (Innovative Medizinische Forschung) of the University of Muenster, and an unrestricted research grant from Astute Medical. Subjects and their insurers or third party payers will not be billed for research related services. 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 copys, coinsurances, and deductibles.

8.2 Research subject costs

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 responsible institution, 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.

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