Supplementary Online Content 1

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Trial Protocol

TRIAL PROTOCOL

REGIONAL CITRATE VERSUS SYSTEMIC HEPARIN ANTICOAGULATION FOR CONTINUOUS RENAL REPLACEMENT THERAPY IN CRITICALLY ILL PATIENTS WITH ACUTE KIDNEY INJURY.

ACRONYM

RICH-TRIAL

A clinical trial within the Sepsis Competency Network (SepNet)

Sponsored by the German Research Foundation

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Signatures

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III. Synopsis

Study-ID	03-AnIt-14 / UKM14-0066
EudraCT:	2014-004854-33
Title of the clinical trial:	Regional citrate versus systemic heparin anticoagulation for continuous renal replacement therapy in critically ill patients with acute kidney injury (RICH-Trial).
Acronym:	RICH-Trial
Indication:	Critically ill patients with acute kidney injury
Phase:	IV
Type of trial, trial design, methodology:	Multi-centre Clinical Trial Two arms, randomised, open, controlled, parallel-group trial
Number of subjects:	To be assessed for eligibility: n = 68.000 To be allocated to trial: n = 1.450 To be analysed: n = 1260 (per protocol)
Primary trial objective:	Anticoagulation of the extracorporeal circuit is required in continuous RRT (CRRT). To this date, it is not clear which anticoagulant should be used for CRRT. HYPOTHESIS: Regional citrate anticoagulation (RCA) for CRRT in critically ill patients with AKI prolongs filter life span and reduces 90-day all cause mortality by approximately 8% (from 48% to 40%) compared to systemic heparin anticoagulation for CRRT.
Secondary trial objective:	Evaluation of the clinical impact of the intervention on - Length of ICU and hospital stay / 1 year all cause mortality - Renal replacement therapy - Safety of the intervention - Cost analysis of renal replacement therapy

Study endpoints:	Primary endpoint: • Filter life span (hours) • Overall survival in a 90-day follow-up period (90-day all cause mortality) Secondary endpoints: • ICU length-of-stay and hospital length-of-stay • Duration of renal replacement therapy • Bleeding complications • Transfusion requirement • Rate of infection during primary ICU stay • Major adverse kidney events at day 28, 60, 90 and after 1 year • Complications of therapy during study treatment • Recovery of renal function and requirement for hemodialysis after day 28, 60, 90 and 1 year • SOFA Scores at day 1-14, 21 and 28 • 28-day, 60-day and 1-year all cause mortality • Selected laboratory parameters • Cost analysis of renal replacement therapy Other variables: • Surveillance of vital parameters on ICU • Safety laboratory parameters • Adverse events Add-on study: • New Biomarkers of acute kidney injury and mediators modulating pro- and anti-inflammatory mediators will be analysed in the blood and urine collected in different centres
Principal inclusion criteria	1. Critically ill patients with clinical indication for CRRT o Urea serum levels > 150 mg/dl or o Potassium serum levels > 6 mmol/l or o Magnesium serum levels > 4 mmol/l or o Blood pH <7.15 or o Urine production < 200 ml/12 h or anuria or o Organ edema in the presence of AKI resistant to diuretic treatment **Or** Severe acute kidney injury (KDIGO 3-classification) despite optimal resuscitation o Urine output of < 0.3 ml/kg/h for ≥ 24 h and/or o > 3-fold increase of the serum creatinine level compared to the baseline value or o Serum creatinine ≥ 4.0 mg/dl with an acute increase of ≥ 0.5 mg/dl **and** 2. At least one of the following conditions o Sepsis or septic shock o Use of catecholamines (norepinephrine or epinephrine ≥ 0.1 µg/kg/min or norepinephrine ≥ 0.05 µg/kg/min + dobutamine or norepinephrine ≥ 0.05 µg/kg/min + vasopressin or norepinephrine + epinephrine ≥ 0.1µg/kg/min) o Refractory fluid overload: worsening pulmonary edema: PaO_2/FiO_2 < 300 mmHg and/or fluid balance > 10% of body weight)

Principal inclusion criteria (continuation):	3. 18-90 years old **and** 4. Intention to provide full intensive care treatment for at least 3 days **and** 5. Written informed consent of the patient or the legal representatives or the authorized representative or the inclusion due to an emergency situation
Principal exclusion criteria:	1. Patients with increased bleeding risk (e.g. active bleeding from ulcers in the gastro-intestinal tract, hypertension with a diastolic blood pressure higher than 105 mm Hg, injuries (intracranial hemorrhage, aneurysm of brain arteries) of or surgical procedures on the central nervous system if a heparinization with a target aPTT 45-60 s is not allowed by the treating neurologist or neurosurgeon, severe retinopathies, bleeding into the vitreum, ophthalmic surgical procdures or injuries, active tuberculosis, infective endocarditis) 2. Diseases or organ damage related with hemorrhagic diathesis (coagulopathy, thrombocytopenia, severe liver or pancreas disease) 3. Dialysis-dependent chronic kidney insufficiency 4. Need of therapeutic anticoagulation (PTT > 60 s, antiXa > 0.6 IE/ml, INR > 2) 5. Allergic reaction to one of the anticoagulantia or ingredients, Heparin-induced thrombocytopenia 6. AKI caused by permanent occlusion or surgical lesion of both renal arteries 7. AKI caused by (glomerulo)nephritis, interstitial nephritis, vasculitis 8. Do-not-resuscitate order 9. Hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura 10. Persistent and severe lactate acidosis in the context of an acute liver failure and/or shock 11. Kidney transplant within the last 12 months 12. Pregnancy and nursing period 13. Abortus imminens 14. No hemofiltration machine free for use at the moment of inclusion 15. Participation in another clinical intervention trial in the last 3 months 16. Persons with any kind of dependency on the investigator or employed by the sponsor or investigator 17. Persons held in an institution by legal or official ordner
Therapy	After inclusion in the clinical trial, the patients will be treated with continuous renal replacement therapy. <u>Start of renal replacement therapy:</u> Relative indication: **within 24 hours after diagnosis severe AKI (KDIGO stage 3)** Clinical indication: **as soon as possible** **The study intervention is the type of anticoagulation for continuous renal replacement therapy.**

Intervention	Experimental intervention Anticoagulation: regional citrate according to a published protocol (target: ionized calcium level: 0.25 – 0.35 mmol/l post-filter) Control intervention Anticoagulation: systemic heparin (target aPTT: 45-60 sec.) Follow-up per patient: Up to 1 year after randomization. Criteria for the termination of renal replacement therapy: Until sufficient recovery of renal function (urine output > 400ml/24h without the use of diuretics or > 2100ml/24h with the use of diuretics). CRRT should be performed for at least 5 days (if cessation criteria are not fulfilled) until RRT can be switched to a discontinuous technique. Active study participation stops with the end of CRRT. In case of restarting RRT during the primary hospitalization, the patient will get the same type of anticoagulation that was used in the first phase.
Time plan:	Start of the trial: Date of randomization of first patient Time to recruit 4 years Last Follow-up: 1 year after randomization of last patient
Sample size calculation	Power calculations are performed based on the two primary outcomes (i) filter life and (ii) overall survival in a 90-day follow-up period. The primary efficacy analysis is intended to show superiority of regional citrate versus systemic heparin anticoagulation for CRRT in critically ill patients with acute kidney injury. An adaptive design with one interim analysis is established. The global (two sided) significance level is set to alpha=0.05. The mean difference of filter life between the treatment groups based on published data is expected to be at least 5 h in favour of the intervention group ± 27h standard deviation within each group. The expected 90-day mortality rate in the control group is 48%. Differences between treatment groups are considered to be clinically meaningful, if the 90-day mortality rate in the experimental intervention group is 40% or smaller. Follow-up of each patient with respect to the second primary outcome will be 90 days. During this period, an expected number of 10% of living patients is expected to be lost to follow-up. The power regarding the first and second primary outcome is set to 90% and 80%, respectively. This corresponds to a 70% chance that in both primary outcomes a significant result is attained.

Statistical methods:	Efficacy: 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 inferential statistical analyses two-sided significance tests will be applied, 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 analysis and population: Evaluation of the primary outcome parameters filter life and overall survival in a 90-day follow-up period (90-day all cause mortality). Beyond descriptive statistical analyses, in the primary analysis significance tests will be applied that provide confirmatory statistical evidence. An inverse normal method based on an alpha spending function according to O'Brien and Fleming will be used to account for type I error enhancement while performing adaptive interim analyses. 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 regional citrate versus systemic heparin anticoagulation for CRRT on the primary outcome parameters filter life and overall survival in a 90-day follow-up period will be assessed using (two-sided) inverse normal Likelihood Ratio tests based on a multivariable linear and Cox regression model, respectively (global significance level 5%, power 90% and 80%, respectively). If the treatment effect on overall survival is significant, the treatment effect will be estimated by means of the 90-day all cause mortality rate in both treatment groups. Safety: Safety data will be evaluated descriptively, including all trial subjects who were enrolled into the trial, were randomized, and started to receive study treatment (safety population). Results are generally reported by mean parameter estimates and associated 95% confidence intervals. Results will be discussed with the Data and Safety Monitoring Board (DSMB). Secondary endpoints: Statistical analysis of the pre-specified secondary endpoints will be performed with descriptive and inferential statistical methods.
GCP conformance:	The present trial will be conducted in accordance with the valid versions of the trial protocol and the internationally recognized Good Clinical Practice Guidelines (ICH-GCP), including archiving of essential documents. The AMG will be followed.
Financing:	The project is supported by the DFG (ZA 428/10-1)

IV. Abbreviations

abbreviation	Meaning
AE	Adverse Event
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
APACHE	Acute Physiology And Chronic Health Evaluation
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
CRRT	Continuous Renal Replacement Therapy
CRF	Case Report Form
CVVH	Continuous Veno-Venous Hemofiltration
CVVHDF	Continuous Veno-Venous HemoDiaFiltration
DSMB	Data Safety Monitoring Board
GFR	Glomerular filtration rate
ICU	Intensive Care Unit
LKP	Principal Coordinating Investigator (Leiter der klinischen Prüfung)
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
MODS	Multiple Organ Dysfunction Syndrome
PCI	Principal Coordinating Investigator
PEI	Paul-Ehrlich-Institut
RCA	Regional Citrat Anticoagulation
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SOFA-score	Sequential Organ Failure Assessment score
SUSAR	Suspected Unexpected Serious Adverse Reaction

1. Introduction

Acute kidney injury (AKI) is a serious complication of critical illness with an important impact on morbidity and mortality. The ICU prevalence is approximately 36%, mortality rates reach 60% when severe enough to require renal replacement therapy (RRT)[1-3]. Sepsis is the leading cause of AKI, which often manifests as part of the multiple organ dysfunction syndrome (MODS). It represents an independent risk factor as patients die of AKI and not simply with. These facts illustrate that an optimal management of patients with AKI is strongly required.

In the past, AKI has been defined as the loss of renal function over a period of hours to days [3]. Analyses showed that mortality rates are significantly higher in patients with AKI and that even small changes in serum-creatinine (≥ 0.3mg/dl) correlate with worse outcome. Therefore, a consensus-based definition and staging criteria for AKI have been developed [4] and this has been subsequently validated [1,2,5]. In 2012 the AKI workgroup modified these criteria and published the latest version of the AKI classification system: KDIGO criteria [6].

At present, the treatment of patients with AKI is limited to RRT as supportive procedure. It is a key component of modern critical care and has long been used to manage complications associated with AKI, such as electrolyte imbalances, uremia, and fluid overload. Although RRT was established > 20 years ago, clinical practice is variable and several fundamental aspects including type of anticoagulation still remain unclear.

Continuous renal replacement therapy (CRRT) is often preferred to intermittent techniques in order to provide tight control of volume and acid base status, and to manage acute hemodynamic instable patients. However, a major disadvantage of CRRT is the need of continuous anticoagulation to prevent clotting of the extracorporeal circuit. In clinical practice, systemic anticoagulation with heparin is very common. As this can be associated with severe adverse events, such as heparin-induced thrombocytopenia (HIT) or an increased risk of bleeding, regional anticoagulation with citrate was developed to avoid systemic anticoagulation. Citrate chelates calcium and decreases ionized calcium in the circuit. As calcium is a cofactor in the coagulation cascade, thrombin generation is inhibited. Citrate and calcium are partially removed by filtration or dialysis. The remaining citrate is rapidly metabolized if liver function and muscle perfusion are sufficient. As calcium is replaced, systemic effects on coagulation are avoided. However, as citrate is a substrate for buffer as well, its use may cause metabolic derangements e.g. hypocalcemia, hypomagnesemia, metabolic alcalosis and citrate accumulation.

Four studies analyzed the influence of citrate on mortality [7-10]. A recently published work by Schilder et al. [9] presents a multicenter trial with only 136 patients. Analyses reveal no difference in 90-day mortality between the two groups (regional citrate anticoagulation vs. systemic heparin anticoagulation). The study was powered for 360 patients, but discontinued after the recruitment of 139 patients due to slow enrolment caused by the need of therapeutic systemic anticoagulation. Therefore, the significance of this study is limited. Hetzel et al. [7] showed in a multicenter trial with 174 patients that mortality rates per day were similar between the two groups during both treatment and follow-up period (3.1 vs 3.1% and 3.8 vs 3.4%, respectively). In contrast, Oudemans-van Straaten et al. [8] demonstrated in a single-center trial that citrate reduced both hospital and 90-day mortality by 18% (p=0.02), and post-hoc analysis showed that this was also valid in the following subgroups: patients after surgery, with sepsis, higher than median SOFA score (11 points), or lower than median age (73 years). In a multivariate model, citrate use was associated with lower 3-month mortality with a hazard ratio of 0.7 (95% CI 0.45-0.98). The authors suggested that the beneficial effects may result from the immunmodulatory effect of citrate [11]. Gritters and colleagues [12] demonstrated that citrate could inhibit dialysis-induced polymorphonuclear cell and platelet degranulation and reduce oxidative stress. In addition, both studies were not powered to test the survival benefit of regional citrate anticoagulation, and the mortality was not the primary endpoint.

Study	Trial type	Number of patients	Outcome (heparin versus citrate
Schilder et al.	prospective, randomized, multicentre trial	n = 136	90-day mortality, 42% vs. 42%, p = 1.0
Hetzel et al.	prospective, randomized multicentre trial	n = 174	study mortality 41% vs. 47%, p = 0.67
Oudemans-van Straaten et al.	randomized controlled single-centre trial	n = 215	90-day mortality, 45% vs. 63%, p = 0.02
Gattas et al.	multicentre, randomized controlled, parallel group trial	n = 212	intra-hospital mortality, 29% vs. 31.4%, p = 0.7

Concerning adverse events, especially major bleedings, five studies showed less bleeding events in the citrate group [7,13-16]. A recent meta-analysis showed a significant difference between the regional citrate and the systemic heparin group, with fewer patients in the citrate group experiencing major bleeding (RR, 0.34; 95% CI, 0.17-0.65) [16]. Another meta-analysis confirmed the reduced bleeding risk and showed that the pooled risk ratio was 0.28 (95% CI

0.15-0.50) [17]. Two trials reported more episodes of alkalosis in the citrate group [14,15], another two reported more such events in the control group [13,16].

Two meta-analyses [16,17] concluded that regional citrate anticoagulation is safe and effective in CRRT as long as appropriate protocols and monitoring mechanisms are in place. Further studies reported on longer circuit survival in the citrate group than in the control group [14,15].

Analyses comparing RRT with citrate and heparin have important limitations and deserve further analyses: 1) there was significant heterogeneity among the randomized controlled trials (e.g. differences in patient characteristics), 2) the number of patients studied was relatively small, 3) some studies adjusted citrate dose based on postfilter ionized calcium levels, whereas other used a fixed dose of citrate in relation to blood flow. Consequently, underdosing of citrate may have had a role in the lack of difference in circuit survival in the trials that did not measure or ensure adequate citrate anticoagulation.

2. Objectives of the clinical trial

2.1. Rationale for the clinical trial

In-hospital mortality in critically ill patients with AKI is often exceeding 50% [3]. Though there is still no consensus for the optimal RRT application in critically ill patients[18].CRRT is often preferred to intermittent techniques in order to provide tight control of volume and acid base status. However, a major disadvantage of CRRT is the need for continuous anticoagulation to prevent clotting of the extracorporeal circuit. In clinical practice, systemic anticoagulation with heparin is common. This can be associated with severe adverse effects, such as heparin-induced thrombocytopenia (HIT) or increased risk of bleeding. Regional anticoagulation with citrate was developed to avoid systemic anticoagulation.

Citrate chelates calcium and decreases ionized calcium in the circuit. As calcium is a cofactor in the coagulation cascade, thrombin generation is inhibited. Citrate and calcium are partially removed by filtration or dialysis [19].The remaining citrate is rapidly metabolized if liver function and muscle perfusion are sufficient [20]. However, as citrate is a substrate for buffer as well, its use may cause metabolic derangements [21].

Circuit life span and metabolic events: Four studies reported significantly longer circuit survival in the citrate group than in the control group [7,14,15]. One study reported no bleeding event in both citrate and control arms [22] and five studies reported less bleeding events in the citrate group [7,8,10 ,13-15], A meta-analysis confirmed the reduced bleeding risk and showed that the pooled risk ratio was 0.28 (95% CI 0.15-0.50) [17]. Two trials reported more episodes of alkalosis in the citrate group [14,15]. whereas other two reported more such events in the control group [8,13]. Hetzel et al. showed that bicarbonate control was comparable between citrate and control group [7]. In the study by Monchi et al.,[15] alkalosis in the citrate group was associated with protocol violation. Systemic hypocalcemia occurred more frequently in the citrate group, which however could be resolved easily and caused no clinically important consequences.

Rate of major bleeding: Several studies evaluated any incidences of major bleeding [7,8,13-15,22]. A recent meta-analysis showed a significant difference between regional citrate and systemic heparin group, with fewer patients in the citrate group experiencing major bleeding (RR, 0.34; 95% CI, 0.17-0.65) [16]. Subgroup analysis demonstrated that a lower risk of bleeding was recorded in the citrate group compared with the lower-molecular weight heparin group (RR, 0.40; 95% CI, 0.16-0.99) [8]. The number of people needed to receive the treatment before one person would experience a beneficial outcome (number needed to treat) was 6.87 [16].

Mortality: Three studies show conflicting results with regard to the impact of regional citrate anticoagulation (RCA) on mortality, with one reporting improved survival in the RCA arm and the others reporting a non-significant result [7,8,10]. Hetzel et al. [7] showed that mortality rates per day were similar between the two groups during both treatment and follow-up period (3.1 vs. 3.1% and 3.8 vs. 3.4%, respectively). In contrast, Oudemans-van Straaten [8] demonstrated that compared with nadroparin, citrate could reduce both hospital and 3-month mortality by 15% ($P<0.05$), and post hoc analysis showed that this was also valid in subgroups including patients after surgery, with sepsis, higher than median SOFA score (11 points), or lower than median age (73 years). In a multivariate model, citrate use was associated with lower 3-month mortality with a hazard ratio of 0.7 (95% CI 0.45–0.98). The authors suggested that the beneficial effects may result from the immunomodulatory effect of citrate [11,23]. Gritters and colleagues [12] demonstrated that citrate could inhibit dialysis-induced polymorphonuclear cell and platelet degranulation and reduce oxidative stress. In addition, both studies were not powered to test the survival benefit of regional citrate anticoagulation, and the mortality was not the primary endpoint. A recently published study by Schilder et al.[9] did not show a mortality difference between the study group (regional citrate anticoagulation) and the control group (systemic heparin anticoagulation). The study was powered for approximately 360 patients on the basis of 28-day mortality. However, enrolment was discontinued after the recruitment of 139 patients because of slow recruitment. Therefore, the significance of this study is limited. Thus, future well-designed studies are needed to clarify the impact of regional citrate anticoagulation on clinical outcome.

Two meta-analyses concluded that regional citrate anticoagulation is safe and effective in CRRT as long as appropriate protocols and monitoring mechanisms are in place [16,17]. However, important limitations of these analyses deserve further attention: 1) there was significant heterogeneity among the randomized controlled trials (e.g. differences in patient characteristics), 2) the number of patients studied was relatively small: only 2 studies included more than 100 participants, 3) some studies adjusted citrate dose based on postfilter ionized calcium levels, whereas others used a fixed dose of citrate in relation to blood flow. Consequently, underdosing of citrate may have had a role in the lack of difference in circuit survival in the trials that did not measure or ensure adequate citrate anticoagulation.

2.1.1. Benefit-risk assessment

Patients included in this trial suffer from critical illness with severe acute kidney injury (AKI) in need of renal replacement therapy (RRT). Independent of this clinical trial RRT needs to be

initiated. Within the scope of RRT, anticoagulation is mandatory since RRT is an extracorporeal procedure.

In the validation group patients receive regionale citrate as anticoagulant, a well known and commonly applied product. In the control group patients receive systemic heparin, a substance routinely used since 1950. Accordingly, both groups receive substances that have been applied for a long time in critically ill patients. Nevertheless, potential adverse effects may occur. But due to the illness severity, critically ill patients are intensively monitored and laboratory analyses are frequently performed. Therefore, and due to the already common use of the substances, potential side effects will be quickly apparent and can be fixed immediately. Established and published protocols for the application and management of both substances are used in this trial .

Patients randomized to the validation group (regional citrate) may have a treatment benefit. Several studies suggest this but the evidence of these analyses is poor und further investigation is needed to confirm this. Since there is a lack of evidence regarding the benefit of citrate, a participation in this trial may not show the desired success and consequently be without any benefit. In all likelihood, patients randomized to the control group (systemic heparin) will not have an essential change or a deterioration of the prospects for treatment compared to non-participation in this trial.

This clinical trial may result in a substantial improvement of the therapy of critically ill patients with severe acute kidney injury with a significant positive impact on morbidity and mortality. A participation in this trial will not influence the therapeutic measures necessary in the treatment of critically ill patients. All the patients receive standard therapy according to the current standards.

In the context of this study, critically ill patients with acute kidney injury will be investigated. These patients suffer from a life-threatening disease requiring renal replacement therapy to replace the kidneys function and treat or avoid life-threatening complications. As the blood gets in contact with extracorporeal surface and the coagulation system might get activated, the blood needs to be anticoagulated during continuous renal replacement therapy. For this purpose, two different therapy regimes are commonly used: systemic heparin and regional citrate anticoagulation. Current evidence suggests that the use of regional citrate anticoagulation is associated with better outcome. This assumption is based on small studies, a definitive verification with a large multicenter trial is essential. As both therapies

(regional citrate anticoagulation and systemic anticoagulation with heparin) are applied in the clinical routine, the additional burdens and predictable risks are exceptional low.

The current therapy will be stopped immediately if the anticoagulant causes any complications (e.g. heparin-induced thrombocytopenia or lactate-induced acidosis during the therapy with citrate). The burdens and predictable risks will be closely supervised by the treating physician.

Based on the insufficient evidence, we think that the use of regional citrate anticoagulation is associated with a longer filter lifetime and a better outcome.

2.2. Primary objective

The primary study endpoints are

- **filter life span** and
- **overall survival in a 90-day follow-up period (90-day all cause mortality).**

The ultimate goal of therapeutic interventions in AKI is to decrease the high mortality associated with this disease. CRRT is commonly used as supportive treatment in critically ill patients with AKI. Filter clotting due to extracorporeal circuit results in discontinuation of RRT with functional reduction and adverse effects on azotaemic control [24]. However, excessive anticoagulation is associated with higher risk for major bleedings reported to occur in 10-50%[16]. Two meta-analyses suggest that regional citrate anticoagulation prolongs circuit life span [16,17]. Improved filter life implicates less off-time, reduced costs, and lower transfusion rates.

Previous studies have selected a variety of endpoints for assessing mortality in AKI including ICU mortality, hospital mortality and mortality at a fixed time-point following discontinuation of renal support. There are, however, methodological difficulties associated with the selection of an endpoint that is less than entirely objective. The decision to discharge a patient from the ICU or from the hospital is not entirely objective and may be affected by issues other than the patient's medical status such as local practice patterns and the use of intermediate (transitional) care facilities. Thus, the criteria for hospital discharge may be somewhat variable and arbitrary between institutions, and even between patients within a single institution.

The use of a time-delimited endpoint obviates many of these issues and has been utilized in prior studies in critically ill patients [25-27]. For example, twenty-eight-day all cause mortality

was the primary end-point in the PROWESS Study, evaluating the efficacy of activated protein kinase C in critically ill patients with sepsis [27]. However, some studies have suggested that a 28-day or 30-day endpoint may miss a significant percentage of total disease-related mortality [28].

Previous studies of AKI support the use of a mortality endpoint between 30 and 60 days. The duration of AKI is usually no more than several weeks, and the majority of mortality associated with AKI is observed within this time frame. In the study by Mehta et al., mean hospital length-of-stay was 17.1 days in patients treated with CRRT and 26.3 days in patients treated with intermittent hemodialysis, with a longer length of stay in survivors than in non-survivors [29]. The mean duration of therapy in the study comparing three doses of CVVH ranged between 11±6 days and 13±8 days [30]. The endpoint of the recently published Randomized Evaluation of Normal versus Augmented Level (RENAL) Replacement Therapy study was 90-day all cause mortality [25].

All of the reported observed mortality in this study occurred prior to day 35, however follow-up was limited to 15-days following discontinuation of RRT [30]. Similarly, in the comparison of daily versus every-other day hemodialysis by Schiffl et al., mean duration of therapy ranged between 9±2 and 16±6 days in the two groups [31]. In a study by Gastaldo et al. comparing two different dialysis membranes, the majority of observed mortality occurred within the first 4 weeks, however mortality rates did not plateau until after day 50 [32].

The use of a 90-day time-point will, however, increase the risk of patients being lost to follow-up following hospital discharge. It is felt, however, that based on the population being studied and the ability to track patient survival using vital registry data, that loss to follow-up will not impact significantly on the ability to track 90-day all cause mortality.

2.3. Secondary and other objectives

Secondary endpoints include:

- ICU length-of-stay AND Hospital length-of-stay

Both ICU and hospital length-of-stay will be defined based on the ICU and acute hospital admissions during patients' randomization. Length-of-stay will be evaluated on the basis of both the mean number of days of ICU/hospital stay following randomization and Kaplan-Meier survival, censored for patient drop out or death. Hospital discharge will be defined as discharge from acute care, whether to acute rehabilitation, transitional care, long-term care or home.

- Duration of renal replacement therapy

The duration of renal support will be defined as the number of days from the initiation of RRT to final dialysis treatment (last data collection 1 year after randomization). Duration of renal support will be censored if the patient is still dialysis dependent at the time of death. Duration of renal support will be evaluated on the basis of both the mean number of days of renal support and Kaplan-Meier survival, censored for patient death. The optimal outcome in AKI is the ability of the patient to return to his or her prior living situation not requiring RRT on an ongoing basis.

- Bleeding complications

Bleeding complications during CRRT and ICU stay (during primary ICU and intermediate care stay) will be defined as major bleeding with transfusion requirement and/or the need of reoperation (hematothorax, relaparotomy and removal of hematoma) and/or new onset of intracranial bleeding without traumatic event.

- Transfusion requirement

Transfusion requirement during CRRT and ICU stay (during primary ICU and intermediate care stay) will be defined as the need of erythrocyte concentrates, fresh-frozen plasma or thrombocyte concentrates.

- Rate of infection during primary ICU.
- Major adverse kidney events at day 28, 60, 90 and after 1 year

Major adverse kidney event (MAKE) will be defined as the composite of death, use of renal replacement therapy and missing renal recovery [33].

- Complications of therapy
- Recovery of renal function and requirement for hemodialysis after day 28, day 60, 1 year

Recovery of renal function will be defined as lack of need for continuing dialysis support, and will be classified as complete recovery, partial recovery or no recovery according to recently published studies [26]. Complete recovery of renal function will be defined as a serum creatinine that is ≤ 0.5 mg/dL greater than baseline. Partial recovery will be defined as a serum creatinine > 0.5 mg/dL greater than baseline but not dialysis-dependent. Patients who remained dialysis dependent at study completion or at time of death will be categorized as having no recovery of renal function (according to the ATN Trial). Multiple studies have

demonstrated that the majority of patients who recover renal function following AKI do so within the first 4 weeks [29-31], justifying the use of the 28-day and 60-day timepoints.

- SOFA Scores at days 1-14, day 21 and day 28

Non-renal organ system failures will be assessed on the basis of SOFA Scores at days 1-14, day 21 and day 28 following randomization for the time of primary ICU stay. Organ failure will be defined as an individual SOFA score ≥ 2. Parameters to be monitored will include the maximum number of non-renal organ failures, the rates of individual non-renal organ-system failures, the time course of non-renal organ failures, and the overall non-renal SOFA score.

- 28-day, 60-day and 1-year all cause mortality
- Selected aboratory parameters (for details see Secondary and other target variables (see also 2.3))
- Cost analysis of renal replacement therapy

An economic analysis will be conducted through documentation of RRT time in days (date of RRT initiation and RRT end) and filter changes while CRRT (documentation of filter type and number of filter changes) to evaluate RRT-specific cost of care.

Safety endpoints include:

- Surveillance of vital parameters on ICU
- Safety laboratory parameters
- Adverse Events

Adverse events (AEs) encountered during the clinical study will be reported in detail in the source documents and reported to the Sponsor (for details see 7). Complications due to RRT during the primary ICU stay will be documented in the eCRF from randomization throughout the clinical conduct up to ICU discharge.

ADD-on study

- To evaluate new biomarkers of AKI, investigate mediators modulating mediators (pro- and anti-inflammatory mediators) and leukocyte function, an add-on study will be performed. Blood and urine samples from recruited patients will be collected on days 0 (day of randomization, before RRT start), 1, 3, 5 and 1 day after CRRT.

3. Organisational and administrative aspects of the trial

3.1. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) composed of independent experts will be set up. It consists of two physicians and a statistician who are not involved in the execution of the trial (see Section 11.2). The task of the DSMB is to oversee the safety of the trial subjects in the clinical trial by periodically assessing the safety and efficacy of the trial therapy, and to monitor the integrity and validity of the collected data and the conduct of the clinical trial.

Throughout this process of surveillance, the DSMB provides the sponsor with recommendations with regard to continuing the trial (e.g. termination or modification) based on the collected data. The data necessary to fulfill this function, are provided by the sponsor as determined by the DSMB. Amongst other datasets, these must include listings providing information on serious adverse events and further variables that the DSMB considers necessary at least every 6 months and when formal interim analyses are conducted.

3.2. Executive committee

A list of members of the Executive committee is given in Appendix 11.3.

3.3. Investigators and trial sites

This clinical trial will be carried out as a multicentre open trial in Germany. If necessary, further qualified trial sites may be recruited to the trial.

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.

3.4. Financing

The clinical trial will be financed by a grant from the German Research Society (Deutsche Forschungsgesellschaft; DFG).

4. Trial conduct

4.1. General aspects of trial design

The Clinical Trial will be performed as an open, controlled, parallelgroup multicentre trial. Eligible patients will be randomized in a ratio of 1:1 to either regional citrate anticoagulation or systemic heparin anticoagulation for CRRT.

Patients who enter the ICU are considered as potential candidates for the study. Patients 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 (for informed consent procedure see ection 4.3). Each patient for who informed consent is obtained or the specific declaration is signed will be assigned a unique patient number. This patient number will be used to identify the patient throughout the study. The patients' 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 two treatment groups.

4.1.1. Time plan

The study comprises three main periods:

- Period from inclusion and randomization to anticoagulation for CRRT
- Treatment period during CRRT
- Follow-up period on days 28, 60, 90 and 1 year after patient enrolment

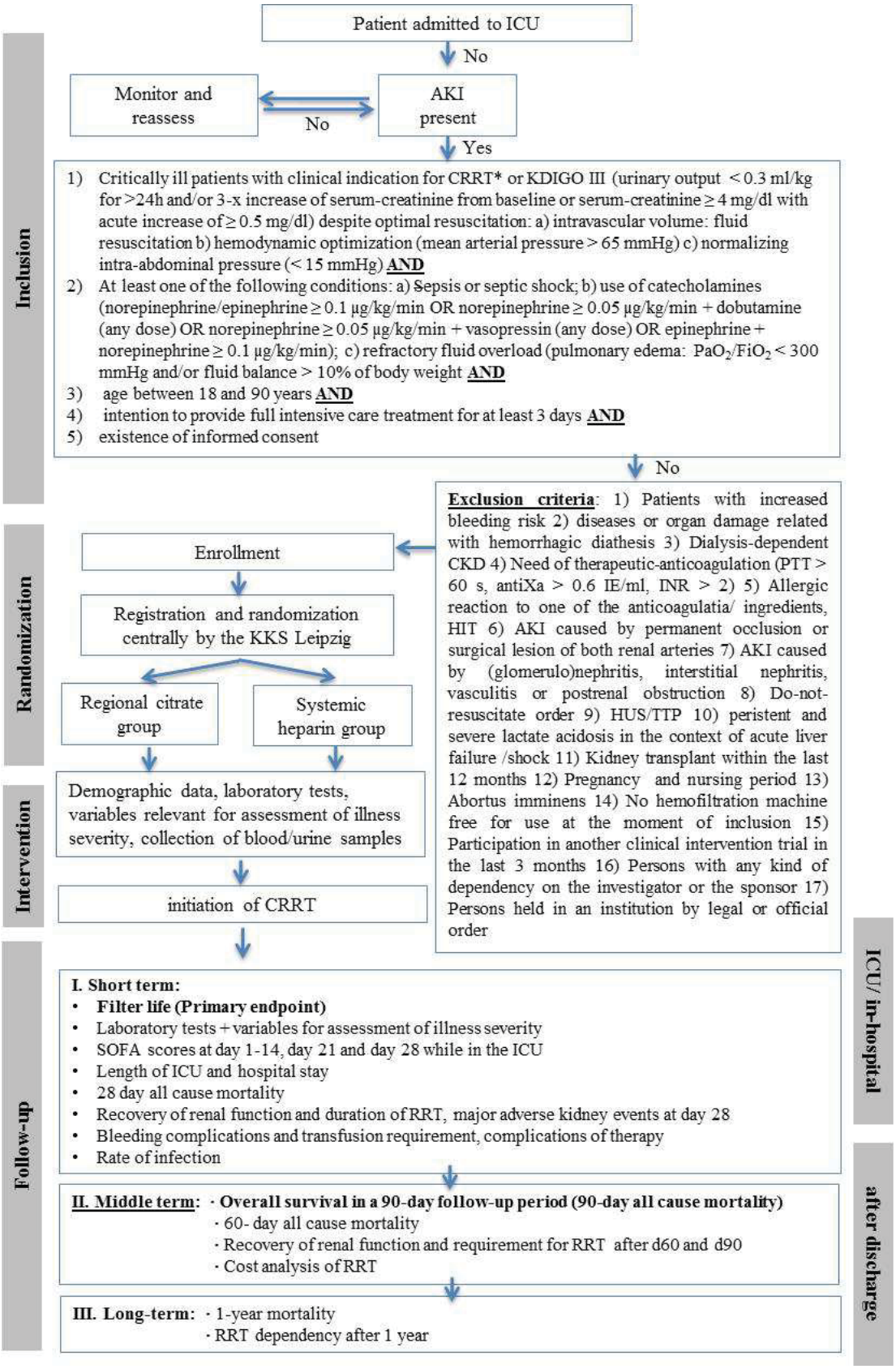
Table 1: Time plan of the trial

Start of the trial	Date of randomization of first patient
Time to recruit	4 years
Last Follow-up	1 year after randomization of last patient

End of the clinical trial

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

Figure 1: Trial flowchart



**Clinical indications for the initiation of CRRT are 1) urea serum levels > 150mg/dl, 2) potassium serum levels > 6mmol/l, 3) magnesium serum levels > 4mmol/l, 4) blood pH < 7.15, 5) urine production < 200ml/12h or anuria, and 6) organ edema in the presence of AKI resistant to diuretic treatment.*[34]

Figure 1 shows the trial work flow. Patients will be identified for recruitment by screening all patients receiving care in the ICUs of participating centers on a daily basis. After obtaining informed consent, the eligible patients will be registered and randomization will be carried out by the Clinical Trial Centre Leipzig (Germany) via an internet based tool. Before initiating CRRT, laboratory tests will be performed and different variables will be documented including demographic data, APACHE II score, SOFA organ-system score, etc (see CRF). In the 'regional citrate group', citrate anticoagulation will be used for CRRT, whereas systemic heparin anticoagulation will be used in the 'systemic heparin group'. Laboratory tests will be analyzed and variables relevant for the assessment of illness severity will be recorded. SOFA Score at different days, length of ICU stay, length of hospitalization, 28-day all cause mortality, recovery of renal function and requirement for hemodialysis after day 28 and day 60, duration of renal replacement therapy, 60-day all cause mortality, 90-day all cause mortality, cost analysis of RRT, rate of infection, transfusion requirement, filter life, and 1 year mortality will be documented at follow-up visits up to one year.

4.2. Discussion of trial design

Multiple pharmacologic interventions have shown promising results in animal models of AKI, however no agents have been demonstrated to be efficacious in clinical practice. As a result, the management of AKI remains primarily supportive, with CRRT serving as the cornerstone of therapy in critically ill patients with severe AKI. To investigate the best anticoagulant for CRRT, we will randomly assign patients with CRRT-dependent AKI to receive either regional citrate or systemic heparin anticoagulation. A placebo group of patients treated with continuous CRRT without any anticoagulation is ethically not acceptable.

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 and no exclusion criteria

For the process of obtaining informed consent from trial subject see 4.3. Randomization will be stratified by study center, SOFA Cardiovascular Organ Failure Score (0-2 versus 3-4), presence or absence of oliguria, and gender. Randomization will be performed centrally by the Clinical Trial Centre Leipzig, in proportion 1:1 using a computerized minimization method with random component [35].

Stratification on the basis of SOFA Cardiovascular Organ Failure Score is necessary for the following reason. A score of 3-4 identifies the subgroup of patients with profound hemodynamic instability, manifested by hypotension requiring vasopressor support [36]. Hypotension has been identified as an independent poor prognostic indicator in studies of AKI; the cardiovascular organ failure being the only organ failure independently associated with mortality by the SOFA score in patients with AKI [37].

Treatment assignment will be accomplished using an internet-based randomization tool. A stratified randomization procedure [35] will be used to generate the treatment assignment within each site in order to achieve the best balance of combinations of treatment, cardiovascular SOFA score level (0-2 or 3-4), presence or absence of oliguria, and gender. Patients will enter the treatment protocol immediately after randomization. The Executive Committee will monitor and review the randomization process during the entire enrollment phase of the study.

4.2.2. Blinding, concomitant medication

Neither the patient nor the study personnel at the treating site will be blinded as to the treatment assignment. However, the primary outcomes (filter life and 90-day all cause mortality) are unaffected by the unblinded trial situation. If adjudication of endpoints (e.g. renal recovery) or complications is required, the individual(s) involved in adjudication will be blinded to treatment assignment.

Since this study is unblinded, there is the potential that the management of aspects of care other than RRT will differ between the two groups. If systematic differences in the management of these “co-interventions” occur, this may introduce bias and either diminish or accentuate the differences between the two groups. This problem is inherent in any unblinded study and is of particular concern in patients with complex comorbidities in which it is not possible to protocolize all aspects of patient management. Prior studies in the critically ill population, such as the ARDS Net trial [38] have demonstrated that it is possible to perform unblinded studies without undue confounding from co-intervention bias.

Several strategies will be employed to minimize the effect of co-intervention bias.

Management of aspects of care that are thought to have a specific impact on outcomes in AKI has been specified. Management of other aspects of care for which there is consensus regarding optimal management of critically ill patients (e.g., ventilator management in ALI/ARDS, diagnosis and management of ventilator-associated pneumonia, and diagnosis and management of sepsis) will be provided in accordance with these standards of care.

Consensus on the management of many other aspects of critically ill patients (e.g., use of pulmonary artery catheters, selection of pressors) does not exist. The management of these aspects of care (e.g., hemodynamic monitoring, selection of vasopressor agents) has not been specified. Variation in management of these parameters, will occur between centers, and should be adjusted for by stratification by site. In addition, these aspects of care will be monitored during the trial to assure that significant differences are not present between groups. Similarly, we will monitor the use of selected pharmacologic therapies, including medications that have been postulated to have a salutary effect in AKI, and medications that are nephrotoxic and may prolong the duration of AKI (e.g., amphotericin, aminoglycosides, cyclosporine, tacrolimus and radiocontrast agents). Diuretic use will also be monitored. The impact of diuretic therapy on the outcome of established AKI is minimal. While diuretic therapy may increase urine output in oliguric patients, there is no evidence that these drugs alter dialysis requirements, renal recovery or survival in AKI [39]

Intention-to-treat analysis will address attrition bias. To prevent publication bias in the 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. Inclusion criteria

1. Critically ill patients with clinical indication for CRRT (clinical decision to use continuous RRT due to hemodynamic instability)
    - Urea serum levels > 150 mg/dl or
    - Potassium serum levels > 6 mmol/l or
    - Magnesium serum levels > 4 mmol/l or
    - Blood pH < 7.15 or
    - Urine production < 200 ml/12 h or anuria or
    - Organ edema in the presence of AKI resistant to diuretic treatment

    Or

    Severe acute kidney injury (KDIGO 3-classification) despite optimal resuscitation
    - Urine output of < 0.3 ml/kg/h for > 24 h and/or

- > 3 fold increase of the serum creatinine level compared to the baseline value or
- Serum creatinine ≥ 4.0 mg/dl with an acute increase of ≥ 0.5 mg/dl

2. At least one of the following conditions
   - Sepsis or septic shock
   - Use of catecholamines (norepinephrine or epinephrine ≥ 0.1 µg/kg/min or norepinephrine ≥ 0.05 µg/kg/min + dobutamine (any dose) or norepinephrine ≥ 0.05 µg/kg/min + vasopressin (any dose) or epinephrine + norepinephrine ≥ 0.1 µg/kg/min)
   - Refractory fluid overload: worsening pulmonary edema: PaO_2/FiO_2 < 300 mmHg and/or fluid balance > 10% of body weight)
3. 18-90 years old
4. Intention to provide full intensive care treatment for at least 3 days
5. Written informed consent of the patient or the legal representatives or the authorized representative or the inclusion due to an emergency situation

4.3.2. Exclusion criteria

1. Patients with increased bleeding risk or an active bleeding due to vascular damage (ulcers in the gastro-intestinal tract, hypertension with a diastolic blood pressure higher than 105 mm Hg, intracranial haemorrhage, injuries (intracranial haemorrhage, aneurysm of brain arteries) or surgical procedures on the central nervous system (when according to neurologists or neurosurgeons a heparinization with target aPTT 45-60 s is not allowed), severe retinopathies, bleeding into the vitreum, ophthalmic surgical procdures or injuries, active tuberculosis, infective endocarditis)
2. Disease or organ damage related with hemorrhagic diathesis (coagulopathy, thrombocytopenia, severe liver or pancreas disease)
3. Dialysis-dependent chronic kidney insufficiency
4. Need of therapeutic anticoagulation (PTT > 60 s, antiXa > 0.6 IE/ml, INR > 2)
5. Allergic reaction to one of the anticoagulatia, ingredients, Heparin-induced thrombocytopenia
6. AKI caused by permanent occlusion or surgical lesion of both renal arteries
7. AKI caused by (glomerulo)nephritis, interstitial nephritis, vasculitis or postrenal obstruction
8. Do-not-resuscitate order
9. Hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura

10. Persistent and severe lactate acidosis in the context of an acute liver failure and/or shock
11. Kidney transplant within the last 12 months
12. Pregnancy and nursing period (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. (due to the intensive care and the severity of the illness, sexual abstinence is warranted))
13. Abortus imminens
14. No hemofiltration machine free for use at the moment of inclusion
15. Participation in another clinical intervention trial in the last 3 months
16. Persons with any kind of dependency on the investigator or employed by the sponsor or investigator
17. Persons held in an institution by legal or official order

4.3.3. Patient information and consent

Patients who enter the ICU and are considered potential candidates for the study, may only participate if signed written informed consent is provided. According to ICH-GCP and according to the applicable national laws, each patient has to be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she has to be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time and without providing reasons.

However, emergency conditions often occur for critically ill patients, most of them are not capable to provide informed consent. **For these unconscious emergency patients the informed consent process has to follow the legal local-specific regulations (in accordance with the instruction of the local ethics commission)** on the basis of the German Civil Code (§ 1902 and § 1904) and on the basis of ther German Drug Law (§ 40 and § 41).

A ***legally authorized representative ("Betreuer")*** 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 treatment of a patient in an emergency situation may not allow any delay and if the legally authorized representative cannot be appointed in a timely manner the informed

consent of the ***authorised representative*** ("*Bevollmächtigter*") can be obtained. **The consent shall represent the subject's presumed will** and may be revoked at any time, without detriment to the subject.

If, in an emergency situation, consent cannot be obtained, the treatment can be started immediately. Consent for continued participation must be obtained as soon as it is possible and reasonable. Every effort of obtaining consent needs to be documented.

If necessary the emergency situation will be confirmed by a declaration of an experienced consultant physician, who is not involved in the study execution and who is independent of the investigational team.

The local established procedure for the inclusion of incapacitated patients should be maintained by the trial centres.

The trial centres have to follow the local established procedure for the inclusion of unconscious patients.

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 being willing to participate in clinical studies. The information and every attempt has to be documented in the patient's medical record. Once the patient regains the capability of providing informed consent he or she needs to be asked for his or her informed consent to continue with the study. In the patient's informed consent is still pending the appointment of the legally authorized representative by the local court and its statement has to be initiated in accordance with the local requirements, if urgent reasons for the assumption existed, that the patient would not be capable to arrange his/her affairs beyond the current treatment.

If an objection is raised by the representative or the patient, the participation will be terminated immediately. The data collection will be terminated at once and the blood samples will be destroyed.

The collected data up to that time may still be used as far as this is necessary to

a) determine the effects of the substance under investigation,

b) ensure, that the legitimate interests of the patients will be not impaired,

c) fulfil the obligation to provide complete authorization dossiers.

The signed informed consent forms and declaration forms of waved informed consent should be filed by the investigator for possible review by inspectors, monitors or for possible future audits where this is permitted and/or required.

Reasons for gender distribution

We expect a gender distribution of (male:female) 70:30 [26]. No patient will be excluded from the study on the basis of gender. Gender will be used for covariate adjustment in the final analysis. A subgroup analysis will be performed according to gender (see section 6.1.4).

4.4. Withdrawal of trial subjects after trial start

Once a patient is included in the study, the investigator will make every reasonable effort to keep the patient in the study.

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 CRRT (see 4.5.2). Patients who withdraw from active study participation will be requested to permit continued data collection for the remainder of the follow-up period.

4.4.2. Individual stop criteria

To avoid an overwhelming activation of the coagulation system with a subsequent occlusion of the extracorporeal circuit anticoagulation is required for continuous renal replacement therapy in critically ill patients with acute kidney injury. In this study, we compare systemic heparin versus regional citrate anticoagulation, both used in the clinical routine.

Systemic heparin anticoagulation needs to be stopped if heparin-induced thrombocytopenia or another contraindication against systemic anticoagulation occurs.

Regional citrate anticoagulation needs to be stopped if persistent lactate-induced acidosis in the context of an acute liver failure or persistent shock or citrate accumulation ($Ca_{2+total}/Ca_{2+ion.} \geq 2.5$) occurs.

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

4.5.1. Closure of trial sites

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 sponsor 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 the 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 sponsor considers that the trial must be discontinued for safety reasons (e.g. on the advice of the DSMB)
- 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 sponsor decides on whether to discontinue the trial in consultation with the PCI, DSMB, Executive Committee and/or statistician.

4.6. Treatment

4.6.1. Treatments to be given

In order to ensure uniformity of treatment among sites and between the regional citrate and systemic heparin group, it is critical that specific protocols for the performance of RRT strictly adhered to.

Modality of RRT

All patients in both groups will be treated using continuous renal replacement therapy.

Start:

Relative indication: within 24 h after achievement of KDIGO stage 3

Clinical indication: as soon as possible

Dose: The delivered dose will be 20-25 ml/kg/hour; prescribed has to be 30 ml/kg/hour [34,40]. Blood flow will be kept above 100 mL/min. The delivered dose of CRRT will be monitored.

Anticoagulation: This is the tested variable (regional anticoagulation with citrate vs. systemic heparin anticoagulation).

Dose of anticoagulation: RCA according to published protocols (target posthemofilter ionized calcium level: 0.25-0.35 mmol/L), systemic heparin (target aPTT: 45-60s).

Cessation of RRT: RRT will be discontinued if renal recovery defined by urine output (> 400 mL/24h without diuretics or 2100 ml/24h with diuretic stimulation) occurs [41].

CRRT should be performed for at least 5 days (if cessation criteria are not fulfilled) until switching to a discontinuous technique. Active study participation stops with the end of CRRT.

In the case of a re-start of continuous replacement therapy during the primary hospitalization, the patient will get the type of anticoagulation that was used during the first treatment.

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. All

medications will be dose adjusted for renal failure and RRT in accordance with standard dosing guidelines.

4.6.2. Description of investigational medicinal product

4.6.2.1. Treatments to be given

The trial sites will use the study medication as article of trade, which will be used routinely in the hospital.

Investigational therapy

Anticoagulation via regional citrate (e.g. Ci-Ca®Dialysate K2/K4, Fresenius Medical Care; Prismocitrate, Gambro).

Control therapy

The control drug for anticoagulation is Heparin (ATC-code: B01AB01) (e.g. Heparine sodium, 25.000 I.U./ 5 ml, B. Braun Melsungen)., a registered solution.

Dosage

Citrate according to published protocols (target posthemofilter ionized calcium level: 0.25-0.35 mmol/L), systemic heparin (target aPTT: 45-60 sec.).

The dose will be documented in the eCRF.

4.7. Efficacy and safety variables

4.7.1. Measurement of efficacy and safety variables

4.7.1.1. Primary target variable

The primary study endpoints are filter life span and overall survival in a 90-day follow-up period (90-day all cause mortality).

4.7.1.2. Secondary and other target variables (see also 2.3)

- **Length of stay in intensive care unit and hospital**

  Information on ICU and hospital stay will be documented. The following will be recorded for each patient:

  - Date and time of admission to hospital and ICU
  - Date and time of discharge from ICU and hospital 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 (primary hospital stay)
- Dates, times and primary reason of all readmissions to ICU and dates and times of discharges from ICU (during the primary hospital stay)
- 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

- **Duration of renal replacement therapy [d]**

  The duration of renal support will be defined as the number of days from initiation of RRT to final dialysis treatment. Duration of renal support will be censored if the patient is still dialysis dependent at the time of death. Duration of renal support will be evaluated on the basis of both the mean number of days of renal support and Kaplan-Meier survival, censored for patient death. The optimal outcome in AKI is the ability of the patient to return to his or her prior living situation not requiring RRT on an ongoing basis.

- **Bleeding complications**
- **Transfusion requirement**
- **Rate of infection during primary ICU stay**
- **Major adverse kidney events at day 28, 60, 90 and after 1 year**
- **Renal replacement therapy data**

  The following data will be collected

  - CVVHDF
    - Hemodiafilter (type and number of changes)
    - Blood flow
    - Prescribed dose
    - Delivered dose
    - Dialysate flow
    - Replacement fluid rate
    - Ultrafiltration rate
    - Hours of therapy per day
    - 24-h effluent volume
  - Complications of therapy
    - First use reaction
    - Hypotension requiring discontinuation of treatment
    - Air embolism
    - Bleeding

  - New onset of serious arrhythmia during treatment
  - Iatrogenic fluid and/or electrolyte disturbance
  - Seizures
  - Catheter insertion complication
- Indications for termination of renal support

- **Recovery of renal function and requirement for hemodialysis**
- **SOFA Scores at day 1-14, 21 and 28**
- **28-day, 60-day and 1-year mortality**
- **Selected laboratory parameters**
  - Urea during study period
  - Ionised Calcium (post-Filter) during CRRT
- **Cost analysis of renal replacement therapy**

4.7.1.3. Safety analysis

- **Surveillance of vital parameters on ICU**
- **Safety laboratory parameters**

  In addition to the routine laboratory parameters, a daily check on Calcium to ionised Calcium ratio and phosphate level is necessary during active study treatment.
- **Incidence of reported adverse events and serious adverse events (including deaths)**

4.7.1.4. Add-on study

An Add-on Study will be performed to evaluate new biomarkers of AKI, to investigate mediators modulating inflammation and to examine leucocyte function. Blood and urine samples from recruited patients will be collected in different centres and analysed.

4.7.1.5. Description of visits

- **Screening, Baseline**
  - Demographic characteristics (year of birth, height, weight, sex, pre-existing medical conditions, long term medication)
  - Inclusion and exclusion criteria
  - Result of randomization
  - Admission diagnosis, source of admission
  - Cause of AKI
  - KDIGO-criteria (why AKI)

- APACHE II
- Hemodynamics (MAP, HR, CVP)
- Catecholamine therapy
- SOFA-Score*
- Fluid balance (last 24 hrs)
- Urinary output (last 24 hrs)
- Safety laboratory test
- Blood and urine sampling for add-on study
- Concomitant nephrotoxic medication

- **Daily visit day 1 until day 14, day 21**
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score*
  - Renal replacement therapy data
  - Complications of RRT
  - Transfusion requirement (erythrocyte concentrates, fresh frozen plasma, thrombocyte concentrates)
  - Fluid balance
  - Urinary output
  - Selected laboratory parameters (during active study treatment)
  - Safety laboratory test
  - Blood and urine sampling for add-on study (randomization, 1,3 and 5 days after RRT initiation, 1 day after RRT cessation)
  - Complications
  - Mortality
  - Stay (ICU, intermediate care, normal ward)
  - Concomitant nephrotoxic medication
  - Serious adverse advents*

- **Day 28**
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score*
  - Renal Replacement Therapy Data
  - Fluid balance
  - Urinary output
  - Safety laboratory test
  - Complications of RRT
  - Serious adverse events*

- **Day 60**
  - Mortality
  - Length of stay (ICU, Hospital)
  - Duration of ventilator support**
  - Number of days of RRT/RRT dependence
  - Economic and utility data of renal replacement therapy
- **Day 90**
  - Mortality
  - Length of stay (ICU, Hospital)
  - Duration of ventilator support**
  - Number of days of RRT/RRT dependence
  - Economic and utility data of renal replacement therapy
- **1-year follow-up**
  - Mortality
  - Length of stay (ICU, Hospital)
  - Duration of ventilator support**
  - Number of days of RRT/RRT dependence
  - Economic and utility data of renal replacement therapy

* SOFA scores and Serious Adverse Events will be evaluated only during ICU stay

** Duration of ventilator support will be documented in hours, intubated or tracheostomised patients are defined as ventilator supported

Table 2: Investigation during the clinical trial

				Days after Randomization				
Visit	**S[1]**	**R[2]**	**B[3]**	**1-14, 21**	**28**	**60**	**90**	**1 year Follow-up**
Inclusion and Exclusion criteria	X							
Randomization		X						
Demography			X					
Admission diagnosis, source of admission			X					
Cause of AKI			X					
APACHE II			X					
Hemodynamics (MAP, HR, CVP)			X	X	X			
Pressors			X	X	X			
SOFA-Score			X	X	X			
KDIGO criteria	X		X					
Renal replacement therapy data Hemodiafilter, Blood flow, Dialysate flow, Replacement fluid rate, Ultrafiltration rate, Hours of therapy, 24-hour effluent volume Complications of therapy (first use reaction, hypotension requiring discontinuation or treatment, air embolism, bleeding, new onset of serious arrhythmia during treatment, iatrogenic fluid and/or electrolyte disturbance, seizures, catheter insertion complication Indications for termination of renal support				X	X			
Filter life				X				
Bleeding complications and transfusion requirement			X	X	X			
Fluid balance / 24h urine volume			X	X	X			
Concomitant Medication Pressors, , amphotericin, aminoglycosides, vancomycin, , radiocontrast agents, diuretics, heparin			X	X	X			
Safety laboratory test Complete blood count, potassium-, sodium-, ionized and total calcium levels, creatinine and BUN and eGFR, pH, bicarbonate, bilirubine			X	X	X			
Infectiology (leukococytes,CRP, PCT, microbiology)			X	X	X			
Add-on study			X	X[4]				
Mortality				X	X	X	X	X
Length of stay (ICU, Hospital)						X		
Duration of ventilator support						X		
Number of days of RRT/RRT dependence						X	X	X
(Serious) adverse events				X	X			
Economic and Utility data							X	

[1] Screening
[2] Randomization
[3] Baseline
[4] RRT d0, d1, d3, d5 and 1 day after CRRT cessation

4.8. Data quality assurance

4.8.1. Monitoring

The trial sites will be monitored to ensure the quality of the collected data. The objectives of the monitoring procedures are to ensure that the trial safety and rights of the trial subjects as a study participant are respected and that accurate, valid and complete data are collected, and that the trial is conducted in accordance with the trial protocol, the principles of GCP and local legislation.

In order to ensure a high degree of data quality, all recruiting clinical centers will be monitored during the recruitment and follow-up period (frequency depending on the site's recruitment). The monitor will examine patient study files including source documents in both clinic (study) files and patients' official site medical records and will also review regulatory/essential documents. Areas of particular concern will be patient informed consent issues, protocol adherence, safety monitoring, IRB reviews and approvals, regulatory documents, patient records and the site operations/investigator involvement. The exact extent of the monitoring procedures is described in a separate monitoring manual.

4.8.2. Audits/Inspections

As part of quality assurance, the sponsor has the right to audit the trial sites and any other institutions involved in the trial. The aim of an audit is to verify the validity, accuracy and completeness of data, to establish the credibility of the clinical trial, and to check whether the trial subjects' rights and trial subjects' safety are being maintained. The sponsor may assign these activities to persons otherwise not involved in the trial (auditors). These persons are allowed access to all trial documentation (especially the trial protocol, case report forms, trial subjects' medical records, drug accountability documentation, and trial-related correspondence).

The sponsor and all trial sites involved undertake to support auditors and inspections by the competent authorities at all times and to allow the persons charged with these duties access to the necessary original documentation.

All persons conducting audits undertake to keep all trial subject data and other trial data confidential.

The investigator must inform the trial coordination immediately about any inspection announced.

4.9. Documentation

All data relevant to the trial are documented immediately after measuring by the investigator responsible in the electronic case report form supplied. Entering data may be delegated to members of the trial team. The CRFs are signed by the investigator.

4.9.1. Data management

The IT infrastructure and data management staff will be supplied by the Clinical Trial Centre Leipzig. The trial database will be developed and validated before data entry based on working instructions. 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.

Discrepancies and implausible values are clarified in writing between the data manager and the trial site. The trial site has to answer these queries without unreasonable delay.

4.9.2. Archiving

All CRFs, informed consent forms and other important trial materials will be archived for at least 10 years in accordance with § 13 Sec 10 of the GCP Ordinance.

5. Ethical and regulatory aspects

5.1. Independent ethics committee

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

5.2. Ethical basis for the clinical trial

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 intensive care therapy. As no pharmacological therapy for AKI exists, the management of AKI remains primarily supportive, with RRT serving as a cornerstone of therapy in patients with severe acute kidney injury. None of the patients in both groups (“citrate” vs. “heparin”) will be exposed to additional risks. Participation in this study will be voluntary. Written informed consent will be obtained (for further details see 4.3.3).

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 or the sponsor. All collected data will be kept confidential. Study protocol, patient information and informed consent have been submitted to the corresponding ethics committee for appraisal. The principal investigator will inform the ethics committee about any changes in the study protocol. 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 (for further details see 4.3.3). The patient must be given enough time and opportunity to decide on participation and to clarify any questions before beginning of the documentation.

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 (see 4.3.3).

The legally authorized representative has to provide the written informed consent or if there is no authorized representative 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 (see Patient information and consent 4.3.3).

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) and applicable legislation (especially the Federal Drug Law (AMG) and the GCP-V). 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 authorized representatives of the sponsor have the right to review trial documentation and the trial subjects' medical records at any time.

5.3. Notification of the authorities, approval and registration

Before starting the clinical trial, all necessary documentation will be submitted to the competent supreme federal authority for Approval (BfArM). The state authorities in each federal state in which the trial will be conducted will also be notified.

Bevor 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. Obtaining informed consent from trial subjects

For the process of obtaining informed consent from trial subject see 4.3.3. The original signed consent form is archived in the investigator site file. Trial subjects receive copies of the written information sheet, confirmation of insurance with conditions, and the signed informed consent form.

All documents handed out to the trial subject and any recruitment advertisements must be submitted for approval before use to the ethics committee. Part of the monitoring activities are to check that the most recent informed consent form was used before the trial subject was enrolled and that it was dated and signed by the trial subject himself or herself or by another person as outlined in 4.3.3.

5.5. Insurance of trial subjects

All trial subjects enrolled are insured under the group insurance contract of the University Hospital Muenster with HDI Gerling (insurance company). The headquarters, policy number and telephone and fax number will be included in the patient information sheet.

5.6. Data protection

The provisions of data protection legislation will be observed. It is assured by the sponsor that all investigational materials and data will be pseudonymized in accordance with data protection legislation.

Trial subjects will be informed that their pseudonymized data will be passed on in accordance with provisions for documentation and notification pursuant to § 12 and § 13 of the GCP Ordinance 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.

6. Statistical methods and sample size calculation

6.1. Statistical analysis plan

Statistical analyses will be performed according to the principles of the ICH-guideline E9 “Statistical Principles for Clinical Trials” using standard statistical software (SAS or SPSS).
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 the primary statistical analysis, the global (two-sided) significance level is set to alpha=0.05. Two primary outcomes are defined. In order to account for multiple testing a hierarchical test procedure is applied. First the null hypothesis of equal filter life in both treatment groups is tested on a (two-sided) significance level alpha=0.05. If and only if this null hypothesis is rejected, subsequently the null hypotheses of equal overall survival in a 90-day follow-up period is tested on a (two-sided) significance level alpha=0.05. Each of the above two-sided hypotheses are decomposed into two one-sided hypotheses on significance level alpha=0.025, respectively. An adaptive design with one interim analysis is established (see Subsection 6.1.5).

6.1.1. Trial populations

The safety population includes all trial subjects who were enrolled into the trial, were randomized, and started to receive study treatment. Safety analyses will be performed including all patients from the safety population.

All efficacy analyses will be conducted on two trial populations:

The primary dataset for the statistical analysis includes all trial subjects enrolled into the trial and randomized (full analysis set). Statistical analyses will be performed according to the intention-to-treat principle (ITT), i.e. all patients are analyzed in the group to which they were randomized.

The secondary dataset is a subset of the primary dataset and includes all trial subjects who were treated without major protocol violations (per-protocol population, PP). I.e. in particular, the included patients have complete 90-day follow-up (complete case analysis).

As described in Section 4.3.3, a few patients enrolled in the study at the time of enrolment are not capable to provide informed consent in participation and a legally authorized representative (“Betreuer”) is not available in a timely manner. These incapacitated patients

are included in the study preliminarily, e.g., based on the informed consent given by an authorized representative ("Bevollmächtigter"). If later on the previous preliminary consent in a patient's participation is revoked, further data collection will be terminated immediately. The patient's data collected up to that time will be processed regardless of the revokement of the patient's participation, as long as the legitimate interests of the patient are not impaired (see Section 4.3.3). I.e., the patient will be included in the trial populations as described above and his/her data will be used in all efficacy and safety analyses, as long as outcome data are available.

6.1.2. Primary target variable

In the primary efficacy analysis the primary dataset (full analysis set) will be utilized and ITT analyses will be performed as described in Subsection 6.1.1. Primary efficacy analysis provides confirmatory statistical evidence.

The treatment effect on filter life will be assessed using a (two-sided) inverse normal Likelihood Ratio test based on a multivariable linear regression model. The treatment effect on overall survival in a 90-day follow-up period will be assessed using a (two-sided) inverse normal Likelihood Ratio test based on a multivariable Cox regression model. Both Likelihood Ratio tests will be performed by building a null model with the factors study center, SOFA Cardiovascular Organ Failure Score (0-2 versus 3-4), presence or absence of oliguria, and gender. An additional factor in the null model accounts for the changes of inclusion/exclusion criteria that are implemented via amendment 1. I.e., the first factor level indicates patients that were recruited before amendment 1 has been implemented and the second factor level indicates patients that were recruited after implementation of amendment 1. The Likelihood Ratio tests are performed by comparing the null model to a model that additionally includes a treatment effect (regional citrate versus systemic heparin anticoagulation for CRRT).

The interim and final statistical analysis of both primary endpoints (filter life and overall survival in a 90-day follow-up period) will be conducted so that the patients included in the interim analysis (stage 1) are not included in the stage 2 analysis, and vice versa. I.e., in the stage 1 and stage 2 statistical analysis, data of two different and independent cohorts of patients are evaluated.

If the treatment effect on overall survival is significant, the treatment effect will be estimated by means of the 90-day all cause mortality rate in both treatment groups.

Beyond the primary ITT analysis of the primary outcomes, sensitivity analyses will be performed, including PP analyses as described in Subsection 6.1.1. Treatment groups are compared using a Cochran-Mantel-Haenszel test.

6.1.3. Secondary target variables

Statistical analysis of pre-specified secondary outcomes will be performed with descriptive and inferential statistical methods. Secondary outcomes include the rate of bleeding and cost effectiveness analysis. The impact of transfusion requirement on survival will be evaluated using Cox regression with transfusion requirement as a time-dependent covariate. In subgroup analyses, surgical and conservatively treated patients will be analyzed separately. Additional exploratory analyses will include model-based analyses, subgroup analyses, and safety analyses.

Results will be discussed with the Data Safety and Monitoring Board. Results are generally reported by mean parameter estimates and associated 95% confidence intervals. Any applied significance tests will be two-sided. Missing values that may arise in efficacy or safety parameters will not be replaced applying any kind of statistical imputation.

6.1.4. Subgroup analyses

A subgroup analysis according to gender will be performed. We expect a gender distribution of (male:female) 70:30 [26].

6.1.5. Interim analysis

An adaptive design with one interim analysis is established, applying the inverse normal method based on an alpha spending function according to O'Brien/Fleming [42]. The two primary outcomes are (i) filter life and (ii) overall survival in a 90-day follow-up period. The interim analysis is conducted at the time when 400 patients have been recruited, using pre-determined weights in the inverse normal method that correspond to the information rate 0.5. According to this determined time frame, the design of the second primary outcome will be adapted. The trial may be stopped for futility (non-binding), if in the interim analysis the local p value of favourable filter live and/or overall survival in a 90-day follow-up period in the intervention group is 0.5 or larger. In the event of important new discoveries, the design of the study may be changed. In particular the sample size of the final analysis will be re-calculated, see section 6.2.

6.2. Sample size calculation

Power calculations are performed based on the two primary outcomes (i) filter life and (ii) overall survival in a 90-day follow-up period. The primary efficacy analysis is intended to show superiority of regional citrate versus systemic heparin anticoagulation for CRRT in

critically ill patients with acute kidney injury.

An adaptive design with one interim analysis is established (see Subsection 6.1.5). The global (two-sided) significance level is set to alpha=0.05. The mean difference of filter life between the treatment groups based on published data is expected to be at least 5h in favour of the intervention group ±27h standard deviation within each group. The expected 90-day mortality rate in the control group is 48% based on recent published multicentre trials investigating the same patient population. Differences between treatment groups are considered to be clinically meaningful, if the 90-day mortality rate in the experimental intervention group is 40% or smaller. Follow-up of each patient with respect to the second primary outcome will be 90 days. During this period an expected number of 10% of living patients is expected to be lost to follow up. The power regarding the first and second primary outcome is set to 90% and 80%, respectively. This corresponds to a 70% chance that in both primary outcomes a significant result is attained. The interim analysis is performed when 400 patients have been recruited in total across both treatment groups. The final analysis is intended to be performed when 1260 patients have been recruited in total across both treatment groups. In the interim analysis the sample size of the second stage of the trial (i.e., the number of patients recruited after the interim analysis) will be determined as follows. The number of patients will be calculated so that the conditional power across both primary endpoints (EP1 and EP2) is 70%:

$Powercond = 1 - ((1-Power_{cond}^{EP1}) + (1- Power_{cond}^{EP2})) = 70\%,$

under the restriction of a maximal total number of 1450 recruited patients. The conditional power $Power_{cond}^{EP1}$ and $Power_{cond}^{EP2}$ will be calculated based on the treatment effects on the first and second primary outcome, that are observed in the interim analysis. Beyond the calculated sample size of the second stage of the trial, the results of the interim analysis will be presented only to the DSMB. Results will be kept confidential to any trial personnel, in order to keep the integrity of the trial.

Power calculations were performed using the ADDPLAN software.

Compliance/ Rate of loss to follow up

Approximately 10% of the recruited patients are expected to be lost to follow-up during the 90-day follow-up period. The dropout process is assumed to follow an exponential distribution.

7. Safety

7.1. Definitions

The trial compares a medicinal product (heparin) and a medical device (citrate). However, the trial is a drug trial, not a medical device trial. Adverse Events are defined according to the Directive 2001/20/EC, the European Detailed Guidance CT 3, corresponding to the relevant German definitions in the GCP Ordinance (GCP-V). To ensure comparability between the trial arms, these definitions are expanded to include cases from the citrate arm. Relevant definitions for legal pharmacovigilance reporting obligations are observed precisely.

7.1.1. Adverse event

An adverse event (AE) is any untoward medical occurrence in a trial subject administered an investigational treatment (medicinal product or medical device) and which does not necessarily has a causal relationship with this treatment.

Thus any new sign, symptom or disease, or clinically significant increase in the intensity of an existing sign, symptom or disease, should be considered as an AE.

Concomitant diseases

The deterioration of a preexisting illness is also an AE in the context of a clinical trial. The following, however, is not regarded as an AE: a preexisting disease, for which a treatment measure was planned before the start of the clinical trial, e.g. admission to hospital as an inpatient. This should be made clear in the trial subject's medical records and should also be documented in the CRF.

Pregnancy

For reasons of drug safety, the occurrence of a pregnancy during the conduct of this trial is to be regarded as an AE. For details of special reporting requirements for pregnancy, see Section 7.3.

7.1.1. Adverse reaction

An adverse reaction is any untoward and unintended response to an investigational treatment (medicinal product or medical device) related to any dose administered.

All AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to the investigational treatment, qualify as adverse reaction. The expression reasonable causal relationship means that there is evidence or argument to suggest a causal relationship.

The definition covers also medication or use errors and uses outside what is foreseen in the protocol, including misuse and abuse of the investigational treatment.

7.1.2. Serious adverse events

A serious AE (SAE) is any untoward medical occurrence that at any dose

1. Results in death,
2. Is life-threatening at the time of the event
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability/incapacity
5. Is a congenital anomaly or birth defect
6. In the opinion of the investigator, fulfils any other criteria similar to 1.– 4.

Inpatient hospitalisation is defined as any stay in hospital on the part of a trial subject that includes at least one night (midnight to 06:00).

Hospitalisation without underlying adverse event is not an SAE, e.g. admission to hospital as an inpatient planned before the first administration of the investigational treatment, for a pre-existing condition that has not worsened. Such a situation must be documented in the proper manner in the trial subject's medical records and eCRF.

For reasons of drug safety, the occurrence of a pregnancy during the conduct of this trial is to be regarded as serious (see 7.3).

7.1.3. Unexpected adverse reaction

"Unexpected" means that the nature, severity or outcome of the adverse reaction is not consistent with the applicable product information for an investigational medicinal product.

The definition does not apply for citrate, which is not a medicinal product.

- Expected ARs are listed in the reference document. The following document has been chosen as reference document for heparin: German Summary of Product Characteristics ('*Fachinformation'*) for *Heparin-Natrium Braun 25.000 I.E./5 ml Injektions-/Infusionslösung*.

- The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.
- Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events.
- An expected adverse reaction with fatal outcome has to be considered as unexpected as long as the fatal outcome is not explicitly mentioned in the reference document.

7.1.4. Suspected unexpected serious adverse reaction

A suspected unexpected serious adverse reaction (SUSAR) is a serious adverse reaction that has been judged to be unexpected.

The definition does not apply for citrate, which is not a medicinal product.

7.2. Documentation and follow-up of adverse events by the investigator

The sponsor and the investigator ensure that all persons involved in the treatment of trial subjects are adequately informed of the responsibilities and actions required when AEs occur. AEs will be documented in the trial subject’s medical records and on the appropriate pages in the eCRF, according to the rules as outlines below.

AEs including SAEs will be recorded from the time the first dose of heparin or citrate is administered (day 1). Documentation on the AE form will be required up to discharge of the ICU. This is justified by the short half-life of heparin. Documentation within endpoint documentation will follow the rules as outlined in 4.7.1.5.

Rule 1

Acute kidney injury (AKI) is a complex complication which is frequently caused by sepsis/septic shock, extended surgical procedures or traumatic events. Severe AKI is associated with a loss of kidney function requiring renal replacement therapy (RRT). Mortality rates can be as high as 60% depending on the underlying disease. Most of the patients need analgosedation and mechanical ventilation due to illness severity. Parameter of other organ function such as liver, gastrointestinal tract and metabolism are almost always changed.

Therefore, death and other AKI-related events will be documented as clinical results. The recording of these results will be carried out by daily documentation of the severity of multi organ dysfunction (SOFA score). The deterioration of underlying diseases will be requested. These data will be included in the safety and efficacy analyses. These study related clinical

results will **only** be documented **as AE** if the investigator **suspects a reasonable causal relationship to the investigational product (heparin or citrate).**

Rule 1 covers:

- Death caused by underlying diseases (e.g.: sepsis /septic shock)
- Cardiovascular events: aggravation of known congestive heart failure, new myocardial infarction after known acute myocardial infarction
- Neurological events: aggravation of intracerebral bleeding, rupture of known intracerebral aneurysm
- Respiratory events: deterioration of the Horowitz index, mechanical ventilation, hypoxia, ARDS, acute pulmonary dysfunction
- Hepatic events: liver failure or liver dysfunction with an acute increase in serum-bilirubine from baseline
- Hematologic events not related to anticoagulation method: DIC, thrombocytosis
- SIRS criteria: tachypnea, hypopnea, leucocytosis, hypothermia, hyperthermia, tachycardia or bradycardia

Rule 2

All patients requiring RRT need a central venous catheter (CVC). This procedures, as well as RRT itself, are associated with typical risks. These risks exist independently from trial participation. All complications due to CRRT will be documented in the CRF.

Corresponding adverse events **only** have to be documented on th**e AE form if the investigator suspects a reasonable causal relationship to heparin or citrate. If not,** they **only** have to be documented on the **AE form, if the event is serious**.

Rule 2 covers:

- CVC related adverse events:
    - Hemorrhage at the site or CVC insertion with requiring of transfusion > 1 unit of packed red blood cells and/or surgical intervention within 12 h following insertion
    - CVC associated bloodstream infection (bacteremia and culture-positive confirmation of the same organism from the dialysis catheter upon removal)
    - Ultrasonographically-confirmed thrombus attributed to CVC
    - Pneumothorax (for catheters placed in the internal jugular or subclavian position)
    - Hemothorax (for catheters placed in the internal jugular or subclavian position)

  - Air embolism
  - Inadvertent arterial puncture at time of CVC insertion
- RRT associated hypotension: drop in blood pressure requiring
  - Initiation of vasopressor during RRT session
  - Need to escalate dose of vasopressor during RRT session
  - Premature discontinuation of RRT session
  - Any other intervention to stabilize blood pressure
- Severe hypophosphatemia < 0.5 mmol/l
- Severe hypokalemia < 3.0 mmol/l
- New arrhythmia developed during dialysis and was not present prior to dialysis:
  - atrial arrhythmia (excluding sinus arrhythmia or sinus tachycardia)
  - ventricular arrhythmia
- New onset of seizures (not present/known prior to dialysis)

Rule 3

All **other adverse events**, **not listed above**, have to be documented as **AE, independently of causal relationship.** This rule explicitly includes the following events:

- Severe hypocalcemia (ionized calcium < 0.9 mmol/l)
- Allergic reaction during RRT (e.g. heparin-induced thrombocytopenia, thrombocytopenia)
- Hemorrhage during dialysis requiring transfusion of >1 unit of packed red blood cells
- Organ failure due to other reasons than sepsis/septic shock (e.g. anaphylaxis, lung embolism)
- Onset for any other new sign, symptom or disease.

Documentation on the AE form includes:

- Diagnosis or Description of AE
  - If possible, a diagnosis rather than a list of signs, symptoms and laboratory abnormalities should be given.
- Date of onset and date of end of AE
- Seriousness (Yes, SAE reported - Yes, exempted from reporting on SAE form - No)
- Severity (graded according to the general Common Terminology Criteria for Adverse Events (CTCAE) v4.03 scale. A Semi-colon indicates ‘or’ within the description of the grade.):

  - Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
  - Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
  - Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
  - Grade 4: Life-threatening consequences; urgent intervention indicated.
  - Grade 5: Death related to AE.
- Causality (Reasonable possibility, No reasonable possibility)
  - Reasonable possibility: there are facts or arguments to suggest a causal relationship
  - No reasonable possibility: time relationship is improbable, and/or another explanation is more likely (e.g. disease or other drugs provide plausible explanation)
- Action taken with investigational treatment (Dose not changed, Dose reduced, Dose increased, Drug withdrawn, Unknown, Not applicable)
- Outcome (Recovered/resolved, Recovering/resolving, Not recovered/not resolved, Recovered/resolved with sequelae, Fatal, Unknown)

All AEs must be followed up until the condition resolves or stabilizes. The investigator should ensure that adequate medical care is provided to a subject for any adverse events. Transferring a patient from ICU to a normal ward ensures medical care.

Note: Incident reporting for citrate

Citrate is marketed as medical device with CE marking of conformity. Incident (‘*Vorkommnis*’) reporting applies for medical devices with CE mark, whether used in a clinical trial or not. Any user of such a device is responsible for fulfilling legal incident reporting requirements to the competent authority. This reporting obligation is not part of this trial.

7.3. Monitoring pregnancies for potential Serious Adverse Events

In order to identify and follow-up on outcome of pregnancy and on any congenital abnormalities, a positive pregnancy test is reportable on an SAE form within the following time frame: a) during active trial participation, b) thereafter while pregnancy probably was already present during active trial participation. The report should be made as soon as the investigator gains knowledge of the event. Follow-up of a pregnancy will be done using specific additional questionnaires. The Safety Desk will supply these trial adapted forms, when required. However, onset of pregnancy during trial participation is very unlikely, due to the intensive care setting. Information will be collected as far as covered by informed consent (e.g., information about the child needs to be covered by consent of the patient's partner, too). When required, the Trial Coordination will check and assure coverage by informed consent.

7.4. Reporting of serious adverse events (SAE) by the investigator

SAEs have to be reported from the time the first dose of heparin or citrate is administered up to discharge of the ICU.

Protocol-specific exceptions to SAE reporting requirements

The following serious adverse events do not require reporting on the SAE form:

- Clinical results, as defined above (Rule 1), **without** a suspected reasonable causal relationship to the investigational product. Documentation of these events is covered by documenting the SOFA score or will be explicitly requested in the CRF.
- Typical events in connection with CVC or RRT, as defined above (Rule 2), **without** a suspected reasonable causal relationship to the investigational product. These events will be documented on the AE form, marked as serious.

All these events will be monitored by the DSMB.

The following serious adverse events require immediate reporting on an SAE form:

- Clinical results, or typical events in connection with CVC or RRT, as defined above, if the investigator suspects a reasonable causal relationship to the investigational product.
- All other serious adverse events, regardless of whether or not the investigator suspects a reasonable causal relationship to the investigational product.

The investigator has to report all immediately reportable SAEs within 24 hours of knowledge by fax on the SAE form to the Safety Desk, Muenster, Germany. Personal data have to be replaced by the unique patient number before forwarding any information.

Safety Desk Contact
Zentrum für Klinische Studien (ZKS) Münster
Von-Esmarch-Straße 62
48129 Münster
Phone: 0251 83 57109
SAE Fax: 0251 83 57112
E-Mail: mssd@ukmuenster.de

Where possible, a diagnosis rather than a list of symptoms should be given. The investigator is responsible for assessment of seriousness, severity and causality of the SAE. The SAE form should be completed with as much information as possible. The investigator should not wait for full details before making the initial report.

Minimal information to be included in any initial report:

1. Unique patient number
2. SAE details
3. Details about administration of investigational treatment
4. Causality assessment of SAE to investigational treatment
5. Reporting investigator

The investigator must fax any relevant follow-up information as soon as possible. In case of death a copy of the autopsy protocol should be provided, if any. The investigator should answer queries on SAE reports as soon as possible.

In case the competent authority or an ethics committee would request details concerning a fatal case, the investigator has to supply the requested information.

In case the investigator gets knowledge of an SAE occurring after the end of the reporting period, for which he suspects a reasonable causal relationship to the investigational treatment, the investigator should also report such an SAE to the Safety Desk. Late SAEs do not have to be documented in the eCRF.

7.5. Assessment of serious adverse events by the sponsor

The Safety Desk will document each SAE, check it and query additionally required information. The Principal Coordinating Investigator, or a named delegate, will review each SAE again for seriousness and relatedness.

The Principal Coordinating Investigator or his delegate will also assess whether a serious adverse reaction to heparin is expected or unexpected (SUSAR) according to the applicable Product Information (see 7.1.3), and whether any SAE might influence the benefit-risk-ratio or might require changes in the conduct of the trial.

7.6. Legal reporting requirements of the sponsor

It is the duty of the Safety Desk to inform the competent authority, the ethics committee, and the participating investigators about all suspected unexpected serious adverse reactions (SUSARs) in accordance with legal requirements and timelines. SUSAR follow-up reports will be submitted, as appropriate.

The Safety Desk will observe SUSAR cross reporting obligations with other trials of the same sponsor investigating any of the same active substances, if any.

The Principal Coordinating Investigator is responsible for the ongoing safety evaluation of the trial. The Safety Desk and the PCI will inform each other immediately about any relevant safety information coming to their knowledge. In case of safety relevant issues (besides SUSAR) which require expedited reporting, the Safety Desk will support the PCI in submitting an appropriate report in due time. This includes issues which might materially alter the current benefit-risk assessment of the investigational treatment, or that would be sufficient to consider changes in the investigational treatment administration or in the overall conduct of the trial, as well as urgent safety measures to protect the subjects against any immediate hazard.

Annual safety reports will be prepared and submitted in accordance with legal requirements (Development Safety Update Report, DSUR). The reports will be trial specific reports covering both investigational treatments citrate and heparin. Data lock point for the report will be the day before the anniversary of the first authorization of the trial by the competent authority. The PCI is responsible for providing the updated benefit-risk assessment and passages requiring medical assessment. The Data Management is responsible for providing information on subject exposure. The Safety Desk is responsible for preparing the template, adding the other parts of the report, finalizing it and submitting it to the competent authority and the ethics committee within 60 days of the data lock point. On request by the competent authority or the ethics committee, additional reports will be prepared.

Details of all AEs will be reported to the competent authority on request.

The Safety Desk will provide information for the DSMB (see 3.1).

8. Use of trial findings and publication

8.1. Reports

8.1.1. Final report

The corresponding authority and the ethics committee will be informed within 90 days that the trial has officially ended.

Within one year of the completion of the trial, the corresponding federal authority and the ethics committee will be supplied with a summary of the final report on the clinical trial containing the principle results.

8.2. 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) [43] .

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 by the sponsor.

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 sponsor in advance, and the sponsor reserves the right to review and comment on such documentation before publication.

9. Amendments to the trial protocol

Changes to the trial protocol 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 Ordinance 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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11. Appendices

11.1. Protocol Agreement Form

Study title: Regional citrate versus systemic heparin anticoagulation for continuous renal replacement therapy in critically ill patients with acute kidney injury (RICH-trial)

Study number: 03-AnIt-14 / UKM 14_0066

Date: 16.06.2017

I confirm that I have read this protocol; I understand it and I will work according to this protocol and to the ethical principles stated in the latest version of the declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable laws and regulations of the country of the study centre for which I am responsible. I will accept the monitor's overseeing of the study.

Name and address:

Signature of Investigator: _____

Date: _____

11.2. Data Safety and Monitoring Board

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11.3. Executive Committee

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1	Univ.-Prof. Dr. med. Alexander Zarbock	Head of the Department of Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Münster, University Münster, Germany
2	Univ.-Prof. Dr. med. G. Marx	Chair of the Department of Intensive Care Medicine, University Hospital Aachen, University Aachen, Germany
3	Akad. Rat Dr. Dipl.stat J. Gerß	Institute of Biostatistics and Clinical Research, University Münster
4	Dipl. Ök./Med. J. Arnholdt	Centre for Clinical Trials, University Münster
5	Prof. Dr. med. D. Kindgen-Milles	Medical director of Critical Care Medicine, University Hospital Düsseldorf, University Düsseldorf, Germany

11.4. Definition / Scores

Definition sepsis and septic shock (new guidelines)

In 2016, the Surviving Sepsis Campaign introduced the new Sepsis3 guidelines. According to this new definition, we will include patients with sepsis and septic shock.

Sepsis is defined as a life-threatening organ dysfunction caused by dysregulated host response to infection. Organ dysfunction can be identified as an acute change in total SOFA score ≥ 2 points consequent to this infection.

The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction.A SOFA score ≥ 2 reflects an overall mortality risk of approximately 10% in general hospital population with suspected infection.

SOFA Score:

PaO_2/FiO_2 (mmHg)	SOFA score
< 400	1
< 300	2
< 200 **and** mechanically ventilated	3
< 100 **and** mechanically ventilated	4

Glasgow coma scale	SOFA score
13–14	1
10–12	2
6–9	3
< 6	4

Mean arterial pressure OR administration of vasopressors required	SOFA score
MAP < 70 mm/Hg	1
dop <= 5 or dob (any dose)	2
dop > 5 OR epi <= 0.1 OR nor <= 0.1	3
dop > 15 OR epi > 0.1 OR nor > 0.1	4

Bilirubin (mg/dl) [µmol/L]	SOFA score
1.2–1.9 [> 20-32]	1
2.0–5.9 [33-101]	2
6.0–11.9 [102-204]	3
> 12.0 [> 204]	4

Platelets×10^3/µl	SOFA score
< 150	1
< 100	2
< 50	3
< 20	4

Creatinine (mg/dl) [µmol/L] (or urine output)	SOFA score
1.2–1.9 [110-170]	1
2.0–3.4 [171-299]	2
3.5–4.9 [300-440] (or < 500 ml/d)	3
> 5.0 [> 440] (or < 200 ml/d)	4

Patients with supected infection who are likely to have prolonged ICU stay or die in the hospital can be promptly identified at the bedside with quick-SOFA.

A sepsis might then be diagnosed if qSOFA is ≥ 2, sonsisting of:

- Respiratory rate ≥ 22/min
- Systolic blood pressure < 100 mmHg
- Altered mentation.

Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. Septic shock is now defined as (both criteria need to be fulfilled):

- clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP≥ 65 mmHg
- serum lactate level > 2 mmol/l (18 mg/dl) despite adequate volume resuscitation.

SOFA-Score

SOFA Score	0	1	2	3	4
Respiration PaO_2/FiO_2	>400	<400	<300	<200	<100
Platelet count (10^3/µl)	>150	<150	<100	<50	<20
Bilirubine (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12
Cardiovacular	No hypotension	MAP <70 mmHg	Dobutamine (any dose)	Norepinephrine/ epinephrine ≤0.1µg/kg/min	Norepinephrine/ epinephrine > 0.1µg/kg/min
GCS	15	13-14	10-12	6-9	<6
Creatinine (mg/dl) or UO (ml/d)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 ≤500	>5.0 <200

APACHE-Score

Points	+4	+3	+2	+1	0	+1	+2	+3	+4
Temp. °C	≥41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤29.9
MAP (mmHg)	≥160	130-159	110-129		70-109		50-69		≤49
HF/min	≥180	140-179	110-139		70-109		55-69	40-54	≤39
AF/min[*1]	≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation	≥500	350-499	200-349		71-199	61-70		55-60	<55
pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Na^+ (mmol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
K^+ (mmol/L)	≥7	6.6-6.69		5.5-5.59	3.5-5.4	1.0-3.4	2.5-2.9		≤2.5
Creatinine (mg/dl) [*2]	≥3.5	2.0-3.4	1.5-1.9		0.6-1.4		<0.6		
Hematocrit (%)	≥60		50-59.9	46-49.9	30 -45.9		20-29.9		<20
Leukocytes (x1000)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
GCS	Points= 15-current GCS								

*1 spontaneous breathing or mechanical ventilation
*2 AKI receives double points

ASA-Score

ASA I	Normal healthy patients
ASA II	Patients with mild systemic disease
ASA III	Patients with severe systemic disease that is limiting but not incapacitating
ASA IV	Patients with incapacitating disease which is a constant threat to life
ASA V	Moribund patients not expected to live more than 24 hrs
ASA VI	A decleared brain-dead patient whose organs are being removed for donor purposes