TRIAL PROTOCOL

EARLY VERSUS LATE INITIATION OF RENAL REPLACEMENT THERAPY IN CRITICALLY ILL PATIENTS WITH ACUTE KIDNEY INJURY.

ACRONYM

ELAIN

Responsible Institution:
University Hospital Muenster
Department of Anaesthesiology,
Intensive Care and Pain Medicine
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Principal Coordinating Investigator:
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Trial protocol code: 05-AnIt-12

Version of 04.04.2013, Version V2-F Amendment 1 incl.

Signatures

Head of the Department of Anesthesiology, Intensive Care and Pain Medicine	Date	Signature Univ.-Prof. Dr. Dr. h.c. H. Van Aken
Principal Coordinating Investigator	Date	Signature Univ.-Prof. Dr. A. Zarbock
Statistician Institute of Biostatistics and Clinical Research, University of Muenster	Datum	Unterschrift Dr. J. Gerß

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II. Synopsis

Responsible Institution:	University Hospital Muenster Department of Anesthesiology, Intensive Care and Pain Medicine Albert-Schweitzer-Campus 1, A1 48149 Muenster Germany
Principal Coordinating Investigator:	**Univ.-Prof. Dr. A. Zarbock** Department of Anesthesiology, Intensive Care and Pain Medicine University Hospital Muenster Albert-Schweitzer-Campus 1, A1 48149 Muenster Germany
Title of the clinical trial:	Early versus late initiation of renal replacement therapy in critically ill patients with acute kidney injury. The ELAIN-trial
Indication:	Critically ill patients with acute kidney injury
Phase:	N/A
Type of trial, trial design, methodology:	Single-centre Clinical Trial Two arm, randomised, open, controlled, parallel-group trial
Number of subjects:	To be assessed for eligibility: n = 8.439 To be allocated to trial: n = 250 To be analysed: n = 250 (intention-to-treat-analysis) n = 230 (other endpoints)
Primary trial objective:	To compare the safety and efficacy of an early initiation of renal replacement therapy (RRT) to late onset of RRT in critically ill patients.
Study endpoints:	Primary endpoint: • 90-day mortality from all causes Secondary endpoint: • Length of ICU stay • Length of hospitalization • Duration of renal replacement therapy • Recovery of renal function by day 28 • SOFA Organ Failure Scores at day 1-14, 21 and 28 • Requirement for hemodialysis after day 60 • 28-day, 60-day and 1-year mortality • 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

Principal inclusion criteria:	Principal inclusion criteria: 1. Critically ill patients with acute kidney injury (KDIGO stage 2-classification) despite optimal resuscitation o Urine output of < 0.5 mL/kg/h for ≥ 12 h and/or > 2fold increase of serum creatinine level compared to the baseline value 2. At least one of the following conditions o Severe sepsis o Use of catecholamines (norepinephrine or epinephrine > 0.1 µg/kg/min) o Refractory fluid overload: worsening pulmonary edema: PaO_2/FiO_2 < 300 mmHg and/or fluid balance > 10% of body weight) o Non-renal SOFA organ system score ≥ 2 3. 18-90 years old 4. Intention to provide full intensive care treatment for at least 3 days 5. Written informed consent Randomization Criteria: 1. Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) > 150 ng/dL Principal exclusion criteria: 1. Pre-existing kidney disease not requiring RRT (GFR < 30 mL/min) 2. Previous renal-replacement therapy 3. AKI caused by permanent occlusion or surgical lesion of the renal artery 4. AKI caused by (glomerulo)nephritis, interstitial nephritis or vasculitis 5. AKI caused by postrenal obstruction 6. Haemolytic-uremic syndrome/thrombotic thrombocytopenic purpura 7. Prior kidney transplant 8. Hepatorenal syndrome 9. AIDS with a CD4 count of < 0.05 x 10^9/L 10. Hematologic malignancy with neutrophils of < 0.05 x 10^9/L 11. No hemofiltration machine free for use at the moment of inclusion 12. Pregnancy 13. Participation in another clinical intervention trial 14. Persons with any kind of dependency on the investigator or employed by the institution responsible or investigator 15. Persons held in an institution by legal or official ordner

Intervention	Experimental intervention Early initiation of RRT will be initiated at stage 2 of the KDIGO classification. Control intervention Late initiation of RRT will be initiated at stage 3 of the KDIGO classification or if absolute indications for RRT are present. Follow-up per patient: Up to 1 year after randomization Duration of intervention per patient: The study intervention consists in onset of RRT. Therefore it stops with initiation of RRT. The RRT will be continued until sufficient recovery of renal function (urine output > 400 ml/24 h without/ 2100 ml/24h with diuretic treatment and creatinine clearance > 20 mL/min)
Time plan:	First patient first visit (FPFV): 1 July 2013 Last patient first visit (LPFV): 30 June 2015 Last patient last visit (LPLV): 30 June 2016 Final study report: 30 June 2017
Statistician:	Dr. J. Gerß Institute of Biostatistics and Clinical Research University of Muenster Albert-Schweitzer-Campus 1, A11 48149 Muenster Germany

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 inductive statistical analyses two-sided significance tests will be applied with a significance level alpha=0.05, appropriately adjusting for multiple testing. The primary efficacy analysis provides confirmative evidence. Further analyses will be regarded explorative (hypothesis generating) and will be interpreted accordingly. All point estimates of parameters of interest will be supplemented by 95% confidence intervals. SAS or SPSS statistical software will be used for all data analyses. Description of the primary efficacy analysis and population: The primary efficacy analysis will include all randomized subjects (full analysis set) and will be performed according to the intent-to-treat principle, i.e. all subjects are analyzed in the group to which they were randomized. Additional sensitivity analyses will be performed according to the per-protocol principle. The effect of early versus late initiation of renal replacement therapy on overall survival in a 90day follow-up period will be compared by using a (two-sided) stratified Log-rank test (global significance level 5%, power 80%). If the difference in 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 recruited study patients (safety population). Results are generally reported by mean parameter estimates and associated 95% confidence intervals. Secondary endpoints: Statistical analyses of the pre-specified secondary endpoints will be performed with descriptive and inductive statistical methods. Type I error enhancement due to multiple significance testing will be accounted for if applicable.
GCP conformance:	The present trial will be conducted in accordance with the valid versions of the trial protocol and the internationally recognised Good Clinical Practice Guidelines (ICH-GCP), including archiving of essential documents.
Participating centres:	University Hospital Münster
Financing:	Financing for the intervention study will be applied for to the Else-Kröner-Fresenius-Stiftung.

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III. Abbreviations

abbreviation	Meaning
AE	Adverse Event
AKI	Acute Kidney Injury
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
CRF	Case Report Form
DMSB	Data Monitoring Safety Board
GFR	Glomerular filtration rate
LKP	Principal Coordinating Investigator (Leiter der klinischen Prüfung)
KDIGO	Kidney Disease Improving Global Outcomes
MODS	Multiple Organ Dysfunction Syndrome
NGAL	Plasma Neutrophil Gelatinase-Associated Lipocalin
PEI	Paul-Ehrlich-Institut
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event

1. Introduction

Historically, acute renal failure has been defined as the loss of renal function developing over a period of hours to days and represents a frequent complication in critically ill patients[1,2]. More recently, a consensus-based definition and staging criteria have been developed [3] and subsequently validated[4-6]. The ICU prevalence of AKI is approximately 36%[4,6]. Sepsis is the leading cause of AKI, which often manifests as part of the multiple organ dysfunction syndrome (MODS)[3,7]. Independent of the underlying disease, AKI is associated with high hospital morbidity and, when severe enough to require renal replacement therapy (RRT), mortality reaches approximately 60%[2]. As patients die of AKI and not „simply" with AKI, it represents a specific and independent risk factor for poor outcome[8,9]. These facts give optimal management of patients with AKI a high priority. RRT has long been used to manage complications associated with AKI, such as electrolyte abnormalities, uremia, and fluid overload. However, the optimal timing of RRT is still unknown. Furthermore, novel biomarkers of acute kidney injury and mediators modulating inflammation will be investigated in an add-on study.

Renal replacement therapy (RRT) is a key component of modern critical care. Although RRT was established >20 years ago, clinical practice is variable[10,11]. Several fundamental clinical aspects remain uncertain, including optimal indication and timing. In the setting of chronic kidney disease, the European Best Practice Guidelines recommend starting chronic RRT when a patient with an estimated glomerular filtration rate (GFR) of <15 mL/min/1.73m^2 has symptoms or signs of uremia, fluid overload or malnutrition in spite of medical therapy or before estimated GFR has fallen to <6 mL/min/ 1.73m^2 in an asymptomatic patient [12]. The situation is very different for patients with acute kidney injury (AKI) where RRT is generally viewed as a type of organ support aimed at achieving metabolic homeostasis and preventing fluid overload and new organ failure. These benefits of RRT must be balanced by potential harm, including risks related to central venous access, infections and anticoagulation [13]. There are several absolute indications where initiation of RRT is considered life saving, i.e. severe hyperkalemia with cardiac compromise, life-threatening metabolic acidosis or uremic pericarditis. However, these conditions are not commonly encountered [10]. Although RRT should be started before the onset of any serious complications of uremia, the optimal indications and triggers remain unclear. Data from the Randomized Evaluation of Normal versus Augmented Level (RENAL) Replacement Therapy study which compared 2 different doses of continuous venovenous hemofiltration (CVVH) in critically ill intensive care unit (ICU) patients with AKI showed that 60% of patients had severe edema when RRT was started, and 40–50% of patients had either a serum creatinine >300 µmol/L (>3.4 mg/dL) or

serum urea >25 mmol/L (>70 mg/dL) [14]. Eight per cent of patients were hyperkalemic (serum K+ >6.5 mmol/L) at the time of RRT.

Studies aimed at determining the optimal time for starting RRT have evaluated various arbitrary cut-offs for serum creatinine, serum urea or urine output, fluid balance, time from ICU admission or duration of AKI and often differentiated between 'early' and 'late' RRT [15-31].

Table 1: Parameters at the time of RRT and subsequent outcome

Parameters at the time or RRT			
Study	Early RRT	Late RRT	Outcome (early versus late RRT)
Bagshow et al. [17] prospective study	Serum creatinine ≤ 309 µmol/L; serum urea ≤ 24.2 mmol/L	Serum creatinine > 309 µmol/L; serum urea > 24.2 mmol/L	Hospital mortality, 71 vs. 53.4%, p< 0.00001
Shiao et al. [20] prospective study	AKI as per RIFLE classification; no AKI or RIFLE Risk	AKI as per RIFLE classification; RIFLE Injury or Failure	Hospital mortality, 43 vs. 75%, p = 0.002
Chou et al. [31] retrospective study	RIFLE-0 or RIFLE-Risk	RIFLE-Injury or RIFLE-Failure	Hospital mortality, 70.8 vs. 69.7%, p > 0.05
Liu et al. [18] prospective study	Serum urea < 27.1 mmol/L	Serum urea > 27.1 mmol/L	Hospital mortality, RR 1.85 with higher urea (95% CI 1.2-3.2)
Gettings et al [19] retrospective study	Serum urea < 21.4 mmol/L	Serum urea > 21.4 mmol/L	Hospital mortality, 61 vs. 80%, p = 0.041
Elahi et al. [21] retrospective study	Urine output < 100 mL in 8 h	Serum urea ≥ 30 mmol/L or serum creatinine ≥ 250 µmol/L or K^+ > 6 mmol/L	Hospital mortality 22 vs. 43%, p< 0.05
Bouman et al. [15] RCT	Urine output < 30 mL/h for 6 h and creatinine clearance < 20 mL/min	Serum urea > 40 mmol/L or K^+ > 6.5 mmol/L or severe pulmonary edema	28-day mortality, 29 vs. 25%, p=0.8
Demirkilic et al. [25] retrospective study	Urine output < 100 mL within 8h post-surgery	Serum creatinine > 440 µmol/L or K^+ > 5.5 mmol/L	Hospital mortality, 23.5 vs. 56%, p = 0.016
Sugahara et al. [16] RCT	Urine output < 30 mL/h for 3 h	Urine output < 20 mL/h for 2 h	14-day mortality, 14 vs. 86%, p < 0.01

In a retrospective study, Gettings et al. [19] assessed the effect of timing of initiation of RRT on outcome in patients with posttraumatic AKI. Serum BUN served as surrogate marker to determine „early" (<60 mg/dl) vs. „late" (>60 mg/dl) initiation of RRT. Survival was 20% in the „late" group compared with 39% in the „early" group (p=0.041). Elahi et al. [21] as well as Demirkilic et al. [25] also reported improved outcome with „early" initiation of RRT. Two prospective multicenter observational studies [17,20] demonstrated that the late initiation of RRT is associated with a higher mortality [17,20], longer hospital stay, longer duration of RRT, and higher dialysis dependence [17]. In the study by Bagshaw et al. [17], timing of RRT was stratified into 'early' and 'late' by median urea and creatinine at the time RRT was started. Timing was also categorized temporally from ICU admission into early (< 2 days), delayed (2-5 days), and late (> 5 days). Renal replacement therapy timing by serum urea showed no significant difference in crude (63.4% for urea ≤ 24.2 mmol/L vs. 61.4% for urea > 24.2 mmol/L; odds

ratio [OR], 0.92; 95% confidence interval [CI], 0.73- 1.15; p = 0.48) or covariate-adjusted mortality (OR, 1.25; 95% CI, 0.91-1.70; p = 0.16). When stratified by creatinine, late RRT was associated with lower crude (53.4% for creatinine > 309 µmol/L vs. 71.4% for creatinine ≤ 309 µmol/L; OR, 0.46; 95% CI, 0.36-0.58; p < 0.0001) and covariate-adjusted mortality (OR, 0.51; 95% CI, 0.37-0.69; P < 0.001). However, for timing relative to ICU admission, late RRT was associated with greater crude (72.8% vs. 62.3% vs. 59%, p < .001) and covariate-adjusted mortality (OR, 1.95; 95% CI, 1.30-2.92; p = 0.001). In the study by Shiao et al. [20], patients were divided into early (RIFLE-0 or -Risk) or late (RIFLE -Injury or -Failure) initiation of RRT by RIFLE criteria. The hospital mortality was significantly elevated in the 'late' group compared to the 'early' group (75% vs. 43%, retrospectively). A randomized controlled trial was performed by Sugahara et al.[16] who evaluated the role of early RRT in 28 patients with AKI post-cardiac surgery. Fourteen patients were started on continuous hemodialysis when their urine volume decreased to <30 mL/h for 3 h. In patients in the 'late' arm (n = 14), RRT was delayed until urine output had fallen to <20 mL/h for 2 h. Survival was significantly better in the group of patients who started RRT earlier. There were no differences between the two groups with respect to age, gender, Acute Physiology and Chronic Health Evaluation (APACHE) II score and serum creatinine level at the time of initiation of RRT. By contrast, another randomized controlled trial to evaluate timing did not reveal any advantages of early initiation of RRT. Bouman et al. [15] allocated patients to three groups: late low-volume hemofiltration (n=36), early high-volume hemofiltration (n=35), and early low-volume hemofiltration (n=35). Survival rate and recovery of renal function were similar in all the three groups. Interestingly, 16% of the patients in the 'late' group showed recovery of renal function without RRT. However, the sample size was too small to sufficiently detect significant differences and the 28-day survival was remarkably higher than that reported in similar studies, suggesting a less critically ill study population (Table 1).

In a multi-center observational study, Liu et al. [18] analyzed data on timing of initiation of RRT based on the median BUN at the time of initiation of RRT. The authors could not find a significant survival benefit in patients receiving early RRT. Unfortunately, their study was limited to patients who had received RRT but excluded patients with AKI that had never received RRT. Chou et al. [31] made the same observation in another retrospective study. This study included 370 patients with AKI and sepsis. Based on the RIFLE classification, the patients were divided into early (RIFLE-0 or -Risk) or late (RIFLE-Injury or -Failure) initiation of RRT. The hospital mortality was not different between the 'early' and the 'late' group (70.8% vs. 69.6%, retrospectively).

2. Objectives of the clinical trial

2.1. Rationale for the clinical trial

Three meta-analyses concluded that earlier institution of RRT in critically ill patients with AKI might be associated with a survival benefit [11,32,33]. However, the studies were heterogeneous and of variable quality with a paucity of randomized controlled trials. Potential benefits of earlier initiation are attributable to more rapid metabolic/uremic control and more effective prevention and management of fluid overload [34]. Some data also suggest that RRT before the onset of severe AKI may attenuate kidney-specific and non-kidney organ injury from acidemia, uremia, fluid overload and systemic inflammation and potentially translate into improved survival and earlier recovery of kidney function [35,36]. The counter-argument is that a strategy of early initiation of RRT might subject patients who would recover renal function with conservative treatment alone, to the potential risks associated with RRT. However, AKI confers a substantial increased risk of death even in patients never treated with RRT [5]. As such, while there may be a risk of "unnecessary" RRT, there could be an even greater risk associated with not providing it. Therefore, a randomized prospective multicenter trial is needed to provide evidence for the best timing of RRT in critically ill patients with AKI. The **primary outcome** of this study is the **overall survival in a 90-day follow-up period (90-day all cause mortality**.

2.2. Primary objective

The primary study endpoint is the **overall survival in a 90-day follow-up period (90-day all cause mortality).**

The ultimate goal of therapeutic interventions in acute kidney injury is to decrease the high mortality associated with this condition. Prior studies have selected a variety of endpoints for assessing mortality in acute kidney injury, 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 [14,37,38]. 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 [38]. However, some studies have suggested that a 28-day or 30-day endpoint may miss a significant percentage of total disease-related mortality [39].

Prior studies of acute kidney injury support the use of a mortality endpoint between 30 and 60 days. The duration of acute kidney injury is usually no more than several weeks, and the majority of mortality associated with acute renal failure 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 [40]. The mean duration of therapy in the study comparing three doses of CVVH by Ronco et al. ranged between 11±6 days and 13±8 days [41]. The endpoint of the recently published Randomized Evaluation of Normal versus Augmented Level (RENAL) Replacement Therapy study was 90-day all cause mortality [14].

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 renal replacement therapy [41]. 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 [42]. 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 [43].

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:

- 28-day all cause mortality
- 60-day all cause mortality
- 1-year all cause mortality
- Recovery of renal function and requirement for hemodialysis after day 28 and day 60

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. Complete recovery of renal function will be defined as a serum creatinine that is no more than 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. Multiple studies have demonstrated that the majority of patients who recover renal function following ARF do so within the first 4 weeks [40-42], justifying the use of the 28-day and 60-day time points.

- Duration of renal support

The duration of renal support will be defined as the number of days from the initiation of renal replacement therapy 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 acute renal failure is the ability of the patient to return to his or her prior living situation not requiring renal replacement therapy on an ongoing basis.

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

Both ICU and hospital length-of-stay will be defined based on the ICU and acute hospital admissions during which the patient was randomized. 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.

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

Non-renal organ system failures will be assessed on the basis of SOFA Organ Failure Scores at days 1-14, day 21 and day 28 following randomization. Organ failure will be defined as an individual SOFA organ failure 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.

Economic Analysis

An economic analysis will be conducted to evaluate:

- Renal replacement therapy-specific cost of care

ADD-on study

- To evaluate new biomarkers of acute kidney injury, 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 and analysed.

3. Organisational and administrative aspects of the trial

3.1. Sponsor

Sponsor : University Hospital Muenster
Albert-Schweitzer-Campus 1, D5
49149 Muenster
Germany

3.2. Principal Coordinating Investigator

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

3.3. Statistics

Statistician: Dr.J. Gerß
Institute of Biostatistics and Clinical Research
University of Muenster
Albert-Schweitzer-Campus1, A11
40149 Muenster
Germany

3.4. Study laboratories and other technical services

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

48149 Muenster

3.5. Central organisation units

Project management: Dr. Carola Wempe
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3.6. Investigators and trial sites

This clinical trial will be carried out as a single centre 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 seperate list. The final version of this list will be attached to the final report of the clinical trial.

3.7. Financing

Financing for the intervention study will be applied for to the Else-Kröner-Fresenius-Stiftung (EKFS).

4. Trial conduct

4.1. General aspects of trial design

The Clinical Trial will be performed as an open, controlled, parallelgroup single-centre trial. Eligible patients will be randomized in a ratio of 1:1 to either early or late RRT.

Patients who enter the ICU and are considered potential candidates for the study may only participate if signed written informed consent is provided or the specific process for unconscious patients in an emergency situation is followed before any study related procedures are initiated (for informed consent procedure see Section 4.3). Each patient for whom informed consent is obtained or the specific declaration is signed and who fulfil the randomization criteria (NGAL > 150 ng/dL) 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 treatment groups.

4.1.1. Time plan

The study comprises three main periods:

- Period from inclusion and randomization to early or late RRT
- Observation period during RRT
- Follow-up period on days 28, 60 and 1 year after patient enrolment

Table 2: Time plan of the trial

First patient first visit (FPFV):	01 July 2013
Last patient first visit (LPFV):	30 June 2015
Last patient last visit (LPLV):	30 June 2016
Final study report:	30 June 2017

<u>End of the clinical trial</u>

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

Figure 1: Trial flowchart

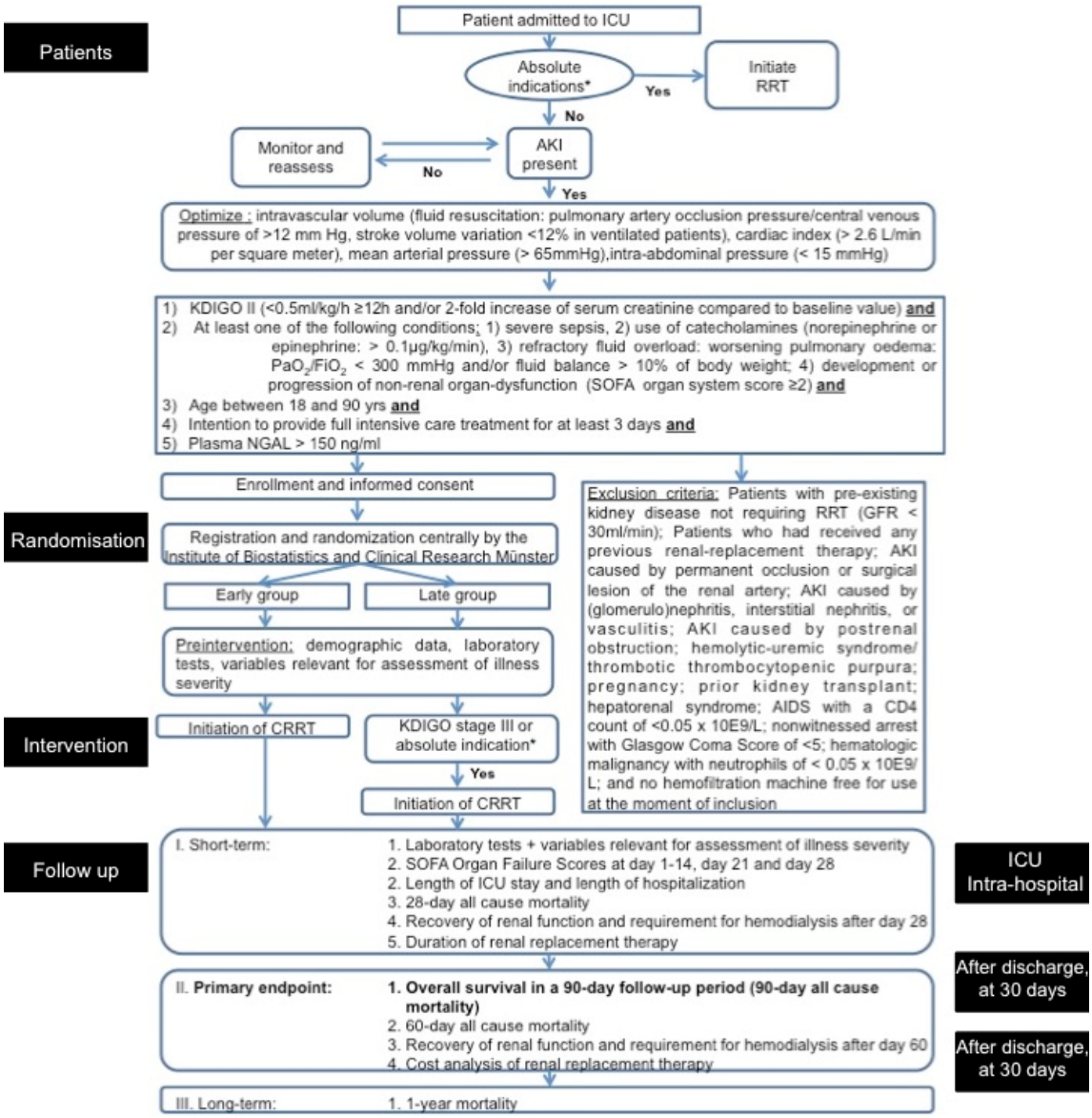

Figure 1 shows the trial workflow. Patients will be identified for recruitment by screening all patients receiving care in the critical care units on a daily basis. After obtaining informed consent and check the randomization criteria, the patient will be registered and randomization will be carried out by the Institute of Biostatistics and Clinical Research Münster. Before initiating RRT, laboratory test will be performed and different variables will be documented including demographic data, APACHE II score, SOFA organ-system score, etc. In the 'early' group, RRT will be initiated immediately after randomization, whereas initiation of RRT in the 'late' group will be started after reaching stage 3 of the KDIGO classification and/or if absolute indications for RRT will be present. Laboratory tests will be analyzed and variables relevant for the assessment of illness severity will be recorded.

SOFA Organ Failure 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 renal replacement therapy, and 1 year mortality will be documented at follow-up visits up to one year. * Absolute indications for the initiation of RRT are 1) urea serum levels > 100mg/dl, 2) potassium serum levels > 6mmol/l and/or ECG abnormalities, 3) magnesium serum levels > 4mmol/l and/or anuria/absence of deep tendon reflexes, 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 [44].

4.2. Discussion of trial design

Renal replacement therapy is the main treatment option for AKI. To investigate the appropriate time point of initiation of RRT, we will randomly assign patients to receive early (KDIGO stage 2) or late (KDIGO stage 3 or if absolute indications for RRT are present) initiation of RRT. A placebo group of patients with acute kidney injury withheld from RRT is ethically not acceptable. The currently accepted, absolute indications for the initiation of RRT are

1) urea serum levels > 100mg/dL,
2) potassium serum levels > 6mmol/L and/or ECG abnormalities,
3) magnesium serum levels > 4mmol/L and/or anuria/absence of deep tendon reflexes,
4) blood pH < 7.15,
5) urine production < 200mL/12h or anuria, and
6) organ edema in the presence of AKI resistant to diurectic treatment).

4.2.1. Randomization

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

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

Randomization assignment (in a 1:1 ratio to the two treatment arms) will be given only to those patients who have NGAL > 150 ng/dL. If the patient is unable to provide informed consent, the legally authorized representative has to provide the written informed consent or in her/his absence a declaration for inclusion in an emergency situation is to be signed by a

consultant physician who is not involved in the study and who is independent of the investigational team. Patient or legally authorized representative informed consent will be obtained as soon as the patient's condition allows it. Randomization will be stratified by SOFA Cardiovascular Organ Failure Score (0-2 versus 3-4) and by the presence or absence of oliguria. Randomization will be performed centrally by the Institute of Biostatistics and Clinical Research Münster, in proportion 1:1 using a computerized minimization method with random component [45].

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 [46]. 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 [47].

The operational definition of AKI for this study requires either a two-fold increase in serum creatinine from baseline and/or the presence of persistent oliguria (< 0.5 ml/kg/h ≥ 12h). Since oliguria is an independent predictor of mortality in AKI [47-49] stratification of randomization based on the presence or absence of oliguria is necessary.

Treatment assignment will be accomplished using an internet-based randomization tool. Patients will be randomized by combination of cardiovascular SOFA score level (0-2 or 3-4) and presence or absence of oliguria. A stratified randomization procedure [45] will be used to generate the treatment assignment within each site in order to achieve the best balance of combination of treatment, cardiovascular SOFA score level (0-2 or 3-4) and presence or absence of oliguria. 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.

4.2.1. Blinding

Neither the patient nor the study personnel at the treating site will be blinded as to the treatment assignment. However, the primary outcome (90-day mortality) is unaffected by the unblended 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 renal replacement therapy 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 co-morbidities 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 [50] have demonstrated that it is possible to perform unblinded studies without undue confounding from cointervention 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 acute kidney injury (e.g., nutrition) have 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.

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

4.3. Selection of trial population

Patients who enter the ICU and are considered potential candidates for the study may only participate if signed written informed consent is provided. 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 country-specific regulations. On the basis of the German Civil Code (§ 1902 and § 1904) and on the basis of ther German Drug Law (§ 40 and § 41) the following informed consent process for unconscious patients in an emergency situation is defined for Germany.

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

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 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. If the patient's informed consent is still pending the appointment of the legally authorized representative it has to be pursued to obtain his or her informed consent. The signed informed consent forms and the declaration forms of waved informed consent should be filed by the investigator for possible review by the University Hospital of Muenster

Reasons for gender distribution

We expect a gender distribution of (male:female) 70 :30 [37]. 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).

4.3.1. Inclusion criteria

1. Akute Kidney Injury: stage 2 of KDIGO classification
   - Urine output of < 0.5 mL/kg/h for ≥ 12 h and/or
   - 2-2.9 -fold increase of the serum creatinine level compared to the baseline value)

   Despite optimal resuscitation
   - Optimizing intravascular volume (fluid resuscitation: pulmonary artery artery occlusion pressure/central venous pressure of > 12 mm Hg)
   - Optimization of cardiac index (> 2.6 L/min/m^2)
   - Hemodynamic optimization (mean arterial pressure > 65 mmHg)
   - Normalizing intra-abdominal pressure (< 15 mmHg)
2. At least one of the following conditions:
   - Severe sepsis
   - Use of catecholamines (norepinephrine or epinephrine > 0.1µg/kg/min)
   - Refractory fluid overload (worsening pulmonary edema: PaO_2/FiO_2 < 300 mgHg and/or fluid balance > 10% of body weight
   - Development or progression of non-renal organ-dysfunction (SOFA organ system score ≥ 2)
3. Age between 18 and 90 years
4. Intention to provide full intensive care treatment for at least 3 days

5. Existence of informed consent

4.3.2. Randomization criteria

1. Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) > 150 ng/dL

4.3.3. Exclusion criteria

1. Patients with pre-existing kidney disease not requiring RRT (GFR < 30 mL/min)
2. Patients who had received any previous renal replacement therapy
3. AKI caused by permanent occlusion or surgical lesion of the renal artery
4. AKI caused by (glomerulo)nephritis, interstitial nephritis or vasculitis
5. AKI caused by postrenal obstruction
6. Haemolytic-uremic syndrome / thrombotic thrombocytopenic purpura
7. Prior kidney transplant
8. hepatorenal syndrome
9. AIDS with a CD4 count of < 0.05 x 10^9/L
10. hematologic malignancy with neutrophils of < 0.05 x 10^9/L
11. No hemofiltration machine free for use at the moment of inclusion
12. Pregnancy (female patients must be surgically sterile or postmenopausal for at least two years or if of childbearing potential must have a negative serum pregnancy test)
13. Participation in another clinical trial
14. Persons with any kind of dependency on the investigator or employed by the responsible institution or investigator
15. Persons held in an institution by legal or official order

4.4. Withdrawal of trial subjects after trial start

Once a patient has beein included in the study the investigator will make every reasonable effort to keep the patient in the study. However, if the investigator has to withdraw a patient from study or if the patient refuses further study participation, a final examination should be

performed. For patients withdrawn from the study, the follow-up information should be obtained, if possible.

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

4.4.1. Procedures for premature withdrawal from treatment during the trial

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

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

4.5.1. Closure of trial sites or premature termination of trial

The sponsor has the right to terminate the study. Reasons, which may require termination, are:

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

4.5.2. Premature termination of trial

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

Premature termination of the trial will be considered if:

- The risk-benefit balance for the trial subject changes markedly
- It is no longer ethical to continue treatment

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

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

4.6. Treatment

4.6.1. Treatments to be given

Early initiation of RRT will be initiated at stage 2 of the KDIGO classification

- urinary output < 0.5mL/kg/h for ≥12h or 2 fold increase of the serum creatinine level compared to the baseline value

Late initiation of RRT will be initiated at the stage 3 of the KDIGO classification or if absolute indications for RRT are present

- KDIGO stage 3:
  - urinary output < 0.3 mL/kg/h for ≥24h and/or
  - >3 fold increase of the serum creatinine level compared to the baseline value and/or
  - serum creatinine of more than or equal to 4.0 mg/dL with an acute increase of at least 0.5 mg/dL
- Absolute indications:
  - urea serum levels > 100mg/dL
  - potassium serum levels > 6mmol/L and/or ECG abnormalities
  - magnesium serum levels > 4mmol/L and/or anuria/absence of deep tendon reflexes
  - blood pH < 7.15
  - urine production < 200mL/12h or anuria
  - organ edema in the presence of AKI resistant to diurectic treatment.

In order to ensure uniformity of treatment and between the early and the late group, it is critical that specific protocols for the performance of renal replacement therapy be strictly adhered to.

Modality of RRT

All patients in both groups will be treated using continuous venovenous hemodiafiltration (CVVHDF). Replacement fluid will be delivered into the extracorporeal circuit before the filter (i.e. predilution), with a ratio of dialysate to replacement fluid of 1:1.

Dose: The effluent flow prescribed will be based on the patient's body weight at the time of randomization and will be 30 mL per kilogram per hour [8,44].Blood flow will be kept above 110 mL per minute. Fluid will be removed by decreasing the flow of the replacement fluid and of the dialysate in equal proportion. The delivered dose of RRT will be monitored based on blood-side urea kinetics.

Anticoagulation: Regional anticoagulation with citrate will be used to prevent circuit clotting.

Cessation of RRT: RRT will be discontinued if renal recovery defined by urine output (> 400 mL/24h without or 2100ml/24h with diuretic treatment) and creatinine clearance (> 20 mL/min) occurs [2].

Additional Treatments

The patient's primary physicians will determine the remainder of patient management consistent with established best practices with the management of critically ill patients. In patients with acute lung injury or the acute respiratory distress syndrome, tidal volume for mechanical ventilation will be approximately 6 mL per kilogram of predicted body weight and adjusted to maintain a peak plateau pressure between 25 and 30 cm of water [50]. Ventilator associated pneumonia will be evaluated and treated in accordance with published clinical practice guidelines and consensus statements [51]. All medications will be dose adjusted for renal failure and renal replacement therapy in accordance with standard dosing guidelines.

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

Follow-up per patient: Up to 1 year after randomization.

4.6.2. Assignment of trial subjects to treatment groups

Patients will be randomized by combinations of cardiovascular SOFA score level (0-2 or 3-4) and presence or absence of oliguria. A stratified randomization procedure [45] will be used to generate the treatment assignment in order to achieve the best balance of combinations of treatment, cardiovascular SOFA score level (0-2 or 3-4) and presence or absence of oliguria.

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 [46]. 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 [47].

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.

4.6.3. Concomitant medication

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. 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 acute kidney injury (e.g., fenoldopam and N-acetylcysteine), 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 on 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 [52].

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 endpoint is the overall survival in a 90-day follow-up period (90-day all cause mortality).

4.7.1.2. Secondary and other target variables

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

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

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

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

  The duration of renal support will be defined as the number of days from the initiation of renal replacement therapy 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 acute renal failure is the ability of the patient to return to his or her prior living situation not requiring renal replacement therapy on an ongoing basis

- **Renal replacement therapy data**

The following data will be collected

- CVVHDF
  - Hemodiafilter
  - Blood flow
  - Dialysate flow
  - Replacement fluid rate
  - Ultrafiltration rate
  - Hours of therapy
  - 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 by day 28 and 60**

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.

- Complete recovery of renal function will be defined as a serum creatinine that is no more than 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.

Multiple studies have demonstrated that the majority of patients who recover renal function following ARF do so within the first 4 weeks [40-42], justifying the use of the 28-day and 60-day time points.

- **SOFA Organ Failure Scores at day 1-14, 21 and 28**

  Non-renal organ system failures will be assessed on the basis of SOFA Organ Failure Scores at days 1-14, day 21 and day 28 following randomization. Organ failure will be defined as an individual SOFA organ failure 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 mortality**
- **Cost analysis of renal replacement therapy**

4.7.1.3. Safety analysis

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

  All adverse events (AEs) encountered during the clinical study will be reported in detail in the source documents. Complications due to RRT will be documented in the eCRF, from the randomization, throughout the clinical conduct and up to the follow-up visit on day 28.

4.7.1.4. Add-on study

To evaluate new biomarkers of acute kidney injury, 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 and analysed.

4.7.1.5. Description of visits

- **Screening, Baseline**
  - Demographic characteristics (date of birth, height, weight, sex)
  - Inclusion and exclusion criteria
  - Result of randomization
  - Admission diagnosis, source of admission
  - Cause of AKI
  - APACHE II
  - SIRS Score
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score
  - KDIGO-criteria
  - Fluid balance
  - Plasma NGAL

- Safety laboratory test
- Blood and urine sampling for add-on study
- Concomitant medication

- **Daily visit day 1 until day 14, day 21**
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score
  - KDIGO-criteria
  - Renal replacement therapy data
  - Fluid balance
  - Safety laboratory test
  - Blood sampling for add-on study (12 hours after randomization, 1,3 and 9 days after randomization
  - Complications
  - Mortality
  - Length of Stay
  - Concomitant medication

- **Day 28**
  - Hemodynamics (MAP, HR, CVP)
  - SOFA-Score
  - KDIGO-criteria
  - Renal Replacement Therapy Data
  - Fluid balance
  - Safety laboratory test
  - Complications of RRT
  - Length of stay (ICU, Hospital)
  - Mortality
  - Duration of ventilator support
  - Number of days of RRT/RRT dependence
  - Serious adverse events

- **Day 60**
  - Mortality
  - Length of stay (ICU, Hospital)
  - 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
- Serious adverse events
- 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

818 **Table 3: Investigations 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					
SIRS score			X					
Hemodynamics (MAP, HR, CVP)	X		X	X	X			
SOFA-Score	X		X	X	X			
KDIGO criteria	X		X	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			
Fluid balance / 24h urine volume	X		X	X	X			
Nutrition Management			X	X	X			
Concomitant Medication Pressors, fenoldopam, N-acetylcysteine, amphotericin, aminoglycosides, cyclosporine, tacrolimus, radiocontrast agents, diuretics			X	X	X			
Plasma-NGAL	X							
Safety laboratory test Complete blood count, potassium-, sodium-, calcium-plasma level, creatinine and BUN and eGFR, pH, bicarbonate, bilirubine	X		X	X	X			
Add-on study			X	X[4]				
Mortality				X	X	X	X	X
Length of stay (ICU, Hospital)				X	X	X	X	X
Duration of ventilator support					X		X	X
Number of days of RRT/RRT dependence					X		X	X
Complications / number of non-renal organ failures					X		X	
Economic and Utility data						X	X	

[1] Screening
[2] Randomization
[3] Baseline
[4] randomization, 1, 3 and 7 days after randomization, 1 day after cessation of RRT

4.8. Documentation

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

4.8.1. Data management

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

Study staff enters the data into the trial database using doubled data documentation, and the data entered is compared. Discrepancies and implausible values are clarified in writing between the data manager and the investigator. These queries need to be answered without unreasonable delay.

4.8.2. Archiving

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

5. Ethical and regulatory aspects

5.1. Independent ethics committee

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

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 renal replacement therapy serving as a cornerstone of therapy in patients with severe kidney injury. None of the patients in both groups (‘early’ and ‘late’ group) will be exposed to additional risks. Participation in this study will be voluntary. Written informed consent will be obtained from patients.

Data collection will be performed pseudonymously and the patient’s name will not appear on any case report form or in any other trial document submitted to the central data management. All collected data will be kept confidential. Study protocol, patient information and informed consent have been submitted to the ethics committees of the University of Münster 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. The patient must be given enough time and opportunity to decide on participation and to clarify any questions before the beginning of documentation of the study.

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

The legally authorized representative has to provide the written informed consent or in his absence a declaration for inclusion in an emergency situation is to be signed by a consultant physician who is not involved in the study and who is independent of the investigational team (see 4.3).

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

5.3. Registration

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

5.4. Insurance of trial subjects

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

5.5. Data protection

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

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

6. Statistical methods and sample size calculation

6.1. Statistical and analytical 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).

A group sequential plan according to O'Brian and Fleming with one interim analysis will be applied. In order to maintain a global significance level alpha=0.05, the interim and the final analysis are performed on local significance levels 0.0052 and 0.0480, respectively. Based on the group sequential plan, in the interim analysis the sample size of the final analysis will be re-calculated applying the Inverse Normal method [53].

In the event of important new discoveries the design of the study may be changed. If an adaptation of the design is necessary, the respective changes may be done according to the conditional rejection error probability method [54,55].

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. The primary efficacy analysis will include all randomized patients (full analysis set) and will be performed according to the intention-to-treat principle, i.e. all patients are analyzed in the group to which they were randomized. Primary efficacy analysis provides confirmatory statistical evidence.

Beyond the primary intention-to-treat analysis of the primary outcome, sensitivity analyses will be performed. In per-protocol analyses only patients without major protocol violations are included. I.e. in particular, the included patients have complete 90-day follow-up (complete case analysis). Treatment groups are compared using a two-sided Cochran-Mantel-Haenszel test.

6.1.1. Trial populations

All analyses will be conducted on two trial populations:

The primary dataset for analysis is derived from the intention-to-treat (ITT) population. This dataset includes all trial subjects enrolled into the trial and randomized.

The secondary dataset for analysis is derived from the per-protocol (PP) population. This dataset includes all trial subjects who were treated according to protocol and reached a defined endpoint in the trial.

6.1.2. Primary target variable

The effect of early initiation of RRT versus late initiation of RRT on overall survival in a 90-day follow-up period will be assessed by comparing the randomized groups with a (two-sided) Log-rank test.

The null hypothesis H_0: $S_0(t)=S_1(t)$ for all t is tested against the (two-sided) alternative H_1: $S_0(t')\neq S_1(t')$ for any t', where $S_0(t)$ and $S_1(t)$ denote the overall survival function in the control group and the experimental treatment group, respectively. If the difference in overall survival is significant, the treatment effect will be estimated by means of the 90-day all cause mortality rate in both treatment groups.

Further sensitivity analyses of the primary outcome are performed in order to address the following issue. Study patients have a different baseline risk profile. This becomes apparent especially in the control group with late initiation of RRT, where three risk groups may be differentiated. There are “high risk” patients who die before the indication for RRT is reached and “low risk” patients who survive the entire 90-day follow-up period and never show the indication for RRT. The remaining “medium risk” patients (as expected about 80% of control patients) show the indication for RRT at a certain time point in the 90-day follow-up period and RRT is initiated. The efficacy of the experimental and the control treatment may differ depending on the patients’ risk profile. Maybe “high risk” patients benefit from experimental treatment with early initiation of RRT substantially compared to control treatment, whereas in “low risk” patients both treatments are equally efficacious. Moreover it is of interest, if both treatments differ in particular in the “medium risk” subgroup, i.e. in those control patients, who actually received RRT, compared to the corresponding patients in the experimental treatment group. In order to address these questions, multivariable statistical analyses are performed using Cox regression. Overall survival is modelled as a function of baseline risk, RRT treatment, and the interaction of baseline risk and RRT treatment. In three different model approaches, RRT treatment is expressed in terms of (i) a time-dependent covariate that impacts survival starting with the time of RRT initiation, (ii) the randomized treatment group, and (iii) the time to initiation of RRT.

6.1.3. Secondary target variables

Statistical analysis of the pre-specified secondary endpoints will be performed with descriptive and inductive statistical methods. Type I error enhancement due to multiple significance testing will be accounted for if applicable. Additional exploratory analyses will include model-based analyses, subgroup analyses, and safety analyses. In safety analyses all study patients will be included (safety population).

Results are generally reported by mean parameter estimates and associated 95% confidence intervals. Any applied significance tests will be two-sided, and will be regarded significant in case p≤0.05. 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 [37].

6.1.5. Interim analysis

A group sequential plan according to O'Brien and Fleming with one interim analysis and a global (two-sided) significance level alpha=0.05 is applied. The expected 90-day mortality rate in the control group with late initiation of RRT is 55%. Differences between treatment groups are to be detected with a power of 80%, if the 90-day mortality rate in the experimental intervention group with early initiation of RRT is 37% or smaller. The expected treatment effect is substantiated by published data and clinical reasoning [41]. The interim analysis is conducted using half of the total number of patients (information rate 0.5). Resulting from these considerations, interim analysis is performed after 53 deaths have been observed across both treatment groups. The final analysis is performed after 106 deaths have been observed across both treatment groups. I.e. assuming an average 50% mortality rate in the 90-day follow-up period, a total number of 106/0.46=230 patients across both treatment groups is included in the final analysis. This correspondends to 250 recruited patients, if an expected number of 5-10% of recruited patients is assumed to be lost to follow up and in the worst case has completely non/evaluable data. Power calculations were performed using the ADDPLAN software.

6.2. Sample size calculation

Power calculations are performed based on the primary endpoint, i.e. the overall survival in a 90-day follow-up period (90-day all cause mortality). The primary efficacy analysis is intended to show superiority of early versus late initiation of RRT in intensive care patients with acute kidney injury. An upper bound of the required number of patients is determined, assuming that in the worst expected case 10% of recruited patients have completely non-evaluable data.

A group sequential plan according to O'Brien and Fleming with one interim analysis and a global (two-sided) significance level alpha=0.05 is applied. The expected 90-day mortality rate in the control group with late initiation of RRT is 55%. Differences between treatment groups are to be detected with a power of 80%, if the 90-day mortality rate in the experimental intervention group with early initiation of RRT is 37% or smaller. The expected treatment effect is substantiated by published data and clinical reasoning. The interim analysis is conducted using half of the total number of patients (information rate 0.5). Resulting from these considerations, interim analysis is performed after 53 deaths have been observed across both treatment groups. The final analysis is performed after 106 deaths have been observed across both treatment groups. I.e. assuming an average 46% mortality rate in the 90-day follow-up period, a total number of 106/0.46=230 patients across both treatment groups is included in the final analysis. This correspondends to 250 recruited patients. Power calculations were performed using the ADDPLAN software.

Compliance / Rate of loss to follow up

Approximately 5-10% of recruited patients are expected to be lost to follow-up during the 90-day follow-up period. In terms of a worst-case approach in the power analysis, dropout patients are assumed to have completely non-evaluable data. Therefore in order to ensure that 240 evaluable patients exist, 260 patients will be recruited. Apart from this worst-case approach pursued in power calculations, in the primary analysis dropout patients in fact are included.

7. Safety

7.1. Definitions of adverse events

7.1.1. Adverse event

An adverse event (AE) is any untoward medical occurrence in a trial subject administered an IMP. There does not necessarily have to be a causal relationship with this treatment.

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 that led to a planned treatment measure 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 (see Section 7.1.2).

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 hospitalisation or prolongation of existing hospitalisation
4. results in persistent or significant disability/incapacity
5. is a congenital anomaly or birth defect (1.-4.: § 3(8) GCP Regulations)
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). Admission to hospital as an inpatient planned before the first admission of the IMP are not SAEs, but must be documented in the proper manner in the trial subject's medical records and CRF (see Section 7.1.1).

7.2. Documentation and follow-up of adverse events

The responsible institution ensures that all persons involved in the treatment of trial subjects are adequately informed of the responsibilities and actions required when AEs occur. Trial

subjects will be asked at each visit whether they have experienced AEs or SAEs. AEs will be documented in the trial subject's medical records.

7.2.1. Documentation of complications

All complications due to RRT will be documented in the CRF.

8. Use of trial findings and publication

8.1. Reports

8.1.1. Final report

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 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)) [56] .

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.

9. Amendments to the trial protocol

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

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

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11. Appendices

11.1. Protocol Agreement Form

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11.1 Protocol Agreement Form

Study title: Early versus late initiation of renal replacement therapy in critically ill patients with acute kidney injury.

Study number: 05-AnIt-12

Date: 04.04.2013; Final version 2.0, Amendment 1 included

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: _____

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