STATISTICAL ANALYSIS PLAN

CRUSADERS & SALADIN

Creep and maintenance fluid Sodium chloride Administration rEduction in cRitically ill adultS: The CRUSADERS randomized controlled trial

Salt balance Detailed Insight – nested substudy: The SALADIN nested substudy

Based on protocol version 1.1

Version: 1.0
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*** RESEARCH PARTICIPANT INFORMATION REMOVED AS PER CLINICALTRIALS.GOV INSTRUCTIONS ***

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Please note that this SAP is based on the standard UZA clinical trial template. UZA does not accept any major modifications. However, the Sponsor and/or the CRO may complete this document to make it consistent with the protocol.

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
(e)CRF	(electronic) Case Report Form
(e)ISF	(electronic) Investigator Site File
(e)TMF	(electronic) Trial Master File
ADH	AntiDiuretic Hormone
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ARDS	Acute Respiratory Distress Syndrome
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CI	Chief Investigator
Co-CI	Co-Chief Investigator
CPAP	Continuous Positive Airway Pressure
CTA	Clinical Trial Authorisation
CTIS	Clinical Trial Information system
CTMS	Clinical Trial Management System
CTR	Clinical Trial Regulation
CTC	Clinical Trial Center
CTU	Clinical Trial Unit
DAOH	Days Alive and Out of Hospital
DAWOLS	Days Alive and WithOut Life Support
DSMB	Data and Safety Monitoring Board
DPO	Data Protection Officer
EC	Ethics Committee
ECW	Extracellular Water
ECG	Electrocardiogram
EFWC	Electrolyte-free water clearance

eGFR	Estimated Glomerular Filtration Rate
ER	Emergency Room
EudraCT	European Clinical trials Database
EudraVIGILANCE	European database for Pharmacovigilance
EVF	Extravascular Fluid
FFM	Fat-Free Mass
FFMH	Fat-Free Mass Hydration
FM	Fat Mass
FPI	First Participant In
FWC	Solute-free water clearance
FWO	Research Foundation Flanders (Fonds voor Wetenschappelijk Onderzoek)
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
GMP	Good Manufacturing Practices
HEFrEF	Heart failure with reduced ejection fraction
HFNC	High Flow Nasal Canula
ICH	International Council on Harmonisation of technical requirements for registration of pharmaceuticals for human use
ICU	Intensive Care Unit
ICW	Intracellular Water
IHD	Intermittent hemodialysis
IMP	Investigational Medicinal Product
IQR	Interquartile Range
ISE	Ion-Selective Electrode
IV	Intravenous
IVF	Intravascular Fluid
KDIGO	Kidney Disease Improving Global Outcomes
LOS	Length Of Stay
LVEF	Left Ventricular Ejection Fraction

MAP	Mean Arterial Pressure
MS	Member State
NRS	Numeric Rating Scale
OR	Operating Room
P/F ratio	PaO2 over FiO2 ratio
PI	Principal Investigator
PM	Project Manager
POCT	Point Of Care Test
PPI	Patient and Public Involvement
RASS	Richmond Analgesia and Sedation Scale
RCT	Randomized Controlled Trial
RMR	Resting Metabolic Rate
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SIADH	Syndrome of Inappropriate ADH Secretion
SAR	Serious Adverse Reaction
SC	Study Coordinator
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SMS-ICU	Simplified Mortality Score for the Intensive Care Unit
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBSA	Total Body Surface Area
TBW	Total Body Water
TMG	Trial Management Group
TSC	Trial Steering Committee
UZA	Universitair Ziekenhuis Antwerpen (Antwerp University Hospital)
VE	Volume Excess

WOCBP	Women of Childbearing Potential
ZAS	Ziekenhuis aan de Stroom (ZAS Network of Hospitals)

VERSION HISTORY LOG

This table should detail the version history for this document. The key elements of the changes to the versions should be detailed here.

Document History		
Version Number	**Date**	**Sections that have been adapted (with short description and name of the person who made the adaptations)**
Version 1	**5 SEPT 2025**	

1. INTRODUCTION

For references we refer to the protocol.

The CRUSADERS trial is a multi-center, two arm, phase IV, 1:1 randomized, blind, low-interventional clinical trial comparing a sodium-chloride reduction strategy (NaCl-poor arm) for fluid creep and maintenance fluids to a standard, isotonic fluid arm (NaCl-rich arm) in ICU-patients.

2. STUDY OBJECTIVES

2.1 Primary objective

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids leads to a better outcome in terms of **mortality and respiratory and renal organ support**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

2.2 Secondary objectives

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids is non-inferior to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions in terms of the incidence of **moderate and severe hyponatremia with a non-inferiority margin for severe hyponatremia (< 125 mmol/L) of 5%, and in the incidence of hyperglycemia**. Additionally, we hypothesize the intervention will result in a lower incidence of **moderate and severe hypernatremia, hyperchloremia and hypoglycemia**.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in a **lower cumulative fluid balance before administration of IV loop diuretics and a delayed and reduced need for IV loop diuretics to manage fluid retention**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **lower incidence of acute kidney injury, a lower new-onset need for renal replacement therapy and a shorter duration renal replacement therapy,** compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **a lower new-onset need for mechanical ventilation and a shorter duration of**

mechanical ventilation, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **a reduced ICU and hospital mortality and shorter ICU and hospital lengths of stay**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **substantially lower sodium and chloride burdens and subsequently lower cumulative fluid balances**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

2.3 Tertiary objective

Not applicable.

2.4 Exploratory objective

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **a lower level of catabolism and muscle wasting**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions.

A nested substudy **SALADIN** (**SA**lt ba**LA**nce **D**etailed **IN**sight) in a targeted 150 patients investigates detailed sodium and chloride balances. Patients in the SALADIN nested substudy will undergo daily urine collections, volume kinetics calculations and bioelectrical impedance analysis and measurements of body weight leading to important additional physiological insights.

We hypothesize that a strategy of reducing the sodium chloride burdens in critically ill adult patients by targeting the sodium and chloride content of fluid creep and maintenance fluids will result in **lower sodium and chloride balances**, compared to a regimen in which fluid creep and maintenance fluids predominantly consist of isotonic solutions. Findings will be correlated with **cumulative fluid balance, solute- and electrolyte-free water clearance, assessments of volume kinetics, bioelectrical impedance analysis and measured body weight**.

3. STUDY DESIGN

3.1 Overview

Investigator-initiated, multi-center, prospective, randomized, double-blind phase IV parallel grouped trial.

3.2 Study population

The CRUSADERS-trials is a multi-center study in four different Belgian mixed ICU's of different sizes in a broad range of critically ill adult patients. To avoid performance bias, all study fluids are blinded.

3.2.1 Inclusion criteria

1. At least 18 years of age
2. Patients who are admitted to the ICU for medical or surgical emergencies, including complications of elective surgery
3. The treating physician expects the patient will still require ICU care in two days, indicating a severe or complex condition at enrollment
4. The patient is expected to receive at least 300 mL of fluid creep or at least 1L of maintenance fluid according to study arm during the first 24h after inclusion

3.2.2 Exclusion criteria

1. A contraindication to hypotonic fluids due to risk of brain edema (including traumatic brain injury, major stroke, intracranial/subarachnoid hemorrhage, meningoencephalitis, intracranial malignancies…), with the timing and clinical judgment left at the discretion of the treating physician.
2. Hyponatremia below 131 mmol/L at admission
3. Admission solely for treatment of fluid accumulation due to cardiac decompensation, without other acute medical conditions requiring ICU-level care. Note: Patients with heart failure as a comorbidity, those on chronic diuretic therapy, or presenting with edema/bilateral lung infiltrates due to other conditions (e.g., sepsis, pneumonia) are not excluded.
4. Patient's death is deemed imminent and inevitable, admission for palliative care or admission solely for organ donation
5. Patient receiving chronic renal replacement therapy
6. Patients referred after a stay of more than 24 hours in another ICU
7. Patients randomized in CRUSADERS before

8. Patient is co-enrolled in an unapproved concomitant ICU-trial or in any trial with an intervention that affects fluid administration or fluid balance

We will not exclude patients enrolled in other interventional trials unless the protocols of the two trials collide (e.g. an intervention that consists of different fluid strategies (either in volume or type) or their interventions have an impact on fluid balance (e.g. diuretic use)). A continuously updated list of ICU-trials approved for co-enrollment, as agreed upon by the TSC, will be maintained.

We will not exclude pregnant patients and do not mandate pregnancy testing women of childbearing potential (WOCBP) in this low-risk, low-intervention context.

Additional exclusion criteria for the SALADIN nested substudy

1. Patients expected to require renal replacement therapy within 24 hours
2. Increased insensible fluid losses: burns, extensive wounds or skin defects or massive diarrhea,…
3. Patients without a urine catheter
4. Patients expected to require bladder irrigation within 24 hours
5. Patients on chronic treatment with loop or thiazide diuretics (including combination preparations)

3.3 Sample size

The sample size calculation was based on the data of the ZAS-sites (where part of the trial will be conducted) and the DEMANDS trial where a 24% relative reduction in mortality and an increase of 6 days in median ventilator-free days was reported in a population of patients with septic shock. We assumed that 15% of patients would die within 90 days while receiving life support, and survivors would receive an average of 7 days of life support, this averages to 70.6 days alive without life support for the NaCl-rich arm. If we estimate that the NaCl-poor arm would reduce 90-day mortality by a 16% relative reduction and yield a 27% decrease in days receiving life support for survivors, this corresponds to an average of 74.2 days alive without live support in the NaCl-poor arm. Based on this, we estimate that **with a power of 80% and a two-sided alpha of 5%, 640 patients are needed** to detect the absolute between-group difference of 3.6 days alive without life support**, including a dropout of 15%**.

These results are based on 3000 simulations using a Mann Whitney U test. The DAWOLS distribution was simulated using Yehya et al. (2019). Mortality is simulated according to a Bernouilli distribution and number of days of life support among survivors according to an exponential distribution. For the exponential distribution the rate parameter λ is 1/mean. This results in the typical distribution with peaks at 0 and 90 we see for DAWOLS. For the NaCl-rich arm a mortality of 15% was used (resulting in a DAWOLS of 0 for 15%) and a mean of 7 days for the exponential distribution of the days of life support for the survivors (DAWOLS are then calculated as 90 minus the number of days of life support). For the NaCl-poor arm a mortality of 12.6% (corresponding to a 16% reduction) is used and a mean of 5.1 days of life support (corresponding to a 27% reduction).

3.4 Randomization

Eligible patients are randomized before the patient's ICU stay reaches the second 12:00 noon. Patients who participate in the SALADIN nested substudy need to be randomized before the first urine is dismissed (usually at the end of the first ICU Day).

Patients are randomised 1:1 using stratified randomization where trial site, invasively mechanically ventilated at time of randomization and surgical admission to ICU are used as stratifiers. Per stratum, permuted block randomization will be used with a variable block size. Full details will be provided in a separate document with restricted access. The allocation sequence list and block sizes are only known by the statistician and data manager, and remain concealed from the investigators until the last patient has completed follow-up.

Patients in the trial are randomized to one of the two study arms, each corresponding to a specific fluid strategy. To maintain operational efficiency and minimize logistical challenges, each study arm is assigned a letter (e.g., A and B) for a one-month period. During this month, patients are randomized to either letter A or B.

After the one-month period, study treatment for patients already randomized to A or B continues unchanged for another 28 days to complete their protocol. However, new patients enrolling in the trial are randomized to two new letters (e.g., C and D), which correspond to the same two treatment arms.

The process continues with two new letters assigned each month to represent a study arm. However, these letters can be reused after a sufficient period has passed, such as when the same letter has not been used in the ICU for at least two months.

This sequential letter assignment process ensures that the study maintains a balance between reducing the risk of unblinding (by regularly changing the labeling of study arms) and avoiding excessive storage demands (by limiting the quantity of fluids required for each lettered arm in the ICU).

3.5 Study schedule

For the study schedule we refer to the protocol.

3.6 Study duration

First randomization is expected in September 2025 and recruitment will run for 30 months (until March 2028).

Patients will be followed up until Day 90 counted from the day of ICU admission (=Day 1). The first ICU Day spans from admission to 8AM the following calendar day; subsequent ICU days run from 8 AM to 8 AM the following calendar day; the day of ICU discharge runs from 8AM to the moment of discharge.

A patient is considered to have completed the trial in case any of the following applies:

- Completion of planned follow-up period
- Loss to follow-up, after 3 attempts to reach the patient. The attempts should be documented in patient record.

- Dead
- Withdrawal of informed consent.

4. STUDY ENDPOINTS

4.1 Primary endpoint

The primary endpoint of the CRUSADERS study is **Days alive and without life support (DAWOLS) at Day 90 counted from the day of ICU admission**.

This is defined as the total number of days alive without mechanical ventilation or renal replacement therapy from ICU admission until day 90.

- Mortality is penalized and receives a value of DAWOLS of zero and is determined at day 90 counted from the day of ICU admission.
- Mechanical ventilation is defined as invasive or non-invasive ventilation (CPAP or with any amount of pressure or volume support but excluding high-flow nasal oxygen). On each ICU Day, the presence of mechanical ventilation is counted when this type of support has been in place. The days following extubation are only counted as ventilator-free days when no re-intubation happens within 48 hours of extubation.
- Renal replacement therapy (RRT) is defined as the use of continuous renal replacement therapy, peritoneal dialysis or intermittent hemodialysis (IHD). The presence of continuous renal replacement therapy is counted when this type of support has been in place during an ICU Day. Periods with up to 3 days between IHD are counted as days with RRT, e.g. if a patient received IHD on day 1, did not receive IHD on days 2–4, and received IHD again on day 5, the patient is considered to have been on RRT for the entire period from day 1 through day 5.
- Vasopressor use is not considered as life support.

4.2 Secondary endpoint

Safety endpoints:

- **Occurrence of moderate and severe hyponatremia**

Moderate hyponatremia is defined as 125-129 mmol/L and severe as <125 mmol/L analyzed as the number of patients with at least one instance of the disturbance.

Each ICU day, routine morning sodium assessment is collected, without its specific timestamp.

To be considered as an instance of hyponatremia, there has to be a sodium level decrease of at least 3 mmol/L compared to the baseline level to account for mild hyponatremia at baseline and for analytical errors. Baseline level is defined as the final measurement obtained prior to randomization and occurrence will be counted from the time of randomization onwards.

The electrolyte levels used for this endpoint are the measurements analyzed in the central lab (ion-specific electrode or ISE). Serum albumine levels, if available on the same blood sample and point-of-care sodium assessments from the blood gas analyzer, performed within 2 hours of the ISE analysis, are recorded to cross-check and to assess potential interference in a sensitivity analysis.

- **Occurrence of moderate and severe hypernatremia**

Moderate hypernatremia is defined as 151-155 mmol/L and severe as >155 mmol/L analyzed as the number of patients with at least one instance of the disturbance.

Each ICU day, routine morning sodium assessment is collected, without its specific timestamp.

To be considered as an instance of hypernatremia, there has to be a sodium level increase of at least 3 mmol/L compared to the baseline level to account for mild hypernatremia at baseline and for analytical errors. Baseline level is defined as the final measurement obtained prior to randomization and occurrence will be counted from the time of randomization onwards.

The electrolyte levels used for this endpoint are all the measurements analyzed in the central lab (ion-specific electrode or ISE). Serum albumin levels, if available on the same blood sample and point-of-care sodium assessments from the blood gas analyzer, performed within 2 hours of the ISE analysis, are recorded to cross-check and to assess potential interference in a sensitivity analysis.

- **Occurrence of moderate and severe hyperchloremia**

Moderate hyperchloremia is defined as 111-115 mmol/L and severe as >115 mmol/L analyzed as the number of patients with at least one instance of the disturbance.

Each ICU day, routine morning chloride assessment is collected, without their specific timestamp.

To be considered as an instance of hyperchloremia, there has to be a chloride level increase of at least 2 mmol/L compared to the baseline level to account for mild hyperchloremia at baseline and for analytical errors. Baseline level is defined as the final measurement obtained prior to randomization and occurrence will be counted from the time of randomization onwards.

The electrolyte levels used for this endpoint are all the measurements analyzed in the central lab (ion-specific electrode or ISE). Serum albumin levels, if available on the same blood sample and point-of-care chloride assessments from the blood gas analyzer, performed within 2 hours of the ISE analysis, are recorded to cross-check and to assess potential interference in a sensitivity analysis.

- **Fluid retention and diuretic use**
    - Time between randomization and the first administration of an intravenous loop diuretic (exact timestamp of the first administration is collected)
    - Proportion of ICU days during which IV loop diuretics were administered (collected without their specific timestamp) counted from randomization
    - Cumulative fluid balance in mL from ICU admission to the morning of the first ICU day on which an IV loop diuretic is prescribed. Daily fluid balance is calculated as total inputs minus total outputs (mL per ICU day) (see Table 2). The cumulative fluid balance is the

sum of all daily fluid balances from ICU admission until the morning of the first ICU day on which an intravenous loop diuretic is prescribed.

- **Occurrence of hyperglycemia and hypoglycemia**
  - Proportion of glycemia > 180 mg/dL over total number of glucose assessments.
  - Mean daily glycemia of every ICU day
  - Occurrence of hypoglycemia < 70 mg/dL analyzed as the number of patients with at least one event (event is seen as occurred if it happens in one single measurement on a given day).

Each day of ICU admission, all glucose assessments on the blood gas analyser are collected, without their specific timestamp. Only assessments after randomization are considered.

- **Acute kidney injury**
  - Occurrence of new-onset stage acute kidney injury (KDIGO stage 2 or 3) from the third ICU day after randomization (number of patients with at least one event)

This metric is calculated as follows: to determine AKI, serum creatinine levels for a given ICU day are based on morning routine sampling, regardless of the exact timing. This means that a creatinine measurement taken at 6 AM on ICU day 2 can still be used to assess AKI on ICU day 3. For urine output criteria, the total urine output over the previous 12 and 24 hours is evaluated at 8 AM each ICU day, when the daily fluid balance is assessed. Note: KDIGO Stage 1 is not assessed in this study. Only KDIGO Stage 2 and Stage 3 criteria are considered for AKI classification. If serum creatinine KDIGO stage and urine output KDIGO stage are different then the highest stage counts for the occurrence.

STAGE	SERUM CREATININE CRITERIA	URINE OUTPUT CRITERIA
KDIGO stage 1	Serum creatinine increases with a factor 1.5 to 1.9 times from baseline* *or* Serum creatinine level increases with at least 0.3 mg/dL in 48 hours	< 0.5 mL/kg/hr for 6–12 hours
KDIGO stage 2	Serum creatinine increases with a factor 2 to 2.9 from baseline*	< 0.5 mL/kg/hr for ≥ 12 hours
KDIGO stage 3	Serum creatinine increases with at least a factor 3 from baseline* *or* Serum creatinine level reaches at least 4 mg/dL *or* Initiation of renal replacement therapy	< 0.3 mL/kg/hr for ≥ 24 hours OR anuria for ≥ 12 hours

Table 1: overview of KDIGO criteria. * Baseline creatinine is the lowest creatinine level in the preceding 3 months, if unavailable in the preceding 6 months, if unavailable the lowest value assessed within the first 48 hours of admission.

- New-onset need for RRT from the second ICU day after the ICU Day of randomization

- Days without renal replacement therapy at D90

This metric is calculated by assigning one point for each day during the 90-day measurement period that the patient is both alive and free from renal replacement therapy (RRT)

Renal replacement therapy is defined as the use of continuous renal replacement therapy, peritoneal dialysis or intermittent hemodialysis. The presence of continuous renal replacement therapy is counted when this type of support has been in place during an ICU Day. Periods with up to 3 days between intermittent hemodialysis are counted as days with RRT, e.g. if a patient received IHD on day 1, did not receive IHD on days 2–4, and received IHD again on day 5, the patient is considered to have been on RRT for the entire period from day 1 through day 5.

- **Mechanical ventilation**
  - New-onset need for mechanical ventilation from the second ICU day after the ICU Day of randomization
  - Ventilator-free days at D90

This metric is calculated as one point for each day during the 90-day measurement period that patients are both alive and free of mechanical ventilation. Mechanical ventilation is defined as invasive or non-invasive ventilation (CPAP or with any amount of pressure or volume support but excluding high-flow nasal oxygen). On each ICU Day, the presence of mechanical ventilation is counted when this type of support has been in place. The days following extubation are only counted as ventilator-free days when no re-intubation happens within 48 hours of extubation.

- **Mortality and length of stay**
  - Days alive and out of hospital at 90 days counted from ICU admission (DAOH90). If a patient was discharged and later readmitted, both admissions count towards the total hospitalization days. Mortality is penalized and receives a value of DAOH of zero. DAOH is determined at day 90 counted from ICU admission.
  - ICU mortality: death occurring at any time while continuously in ICU during the index (initial) ICU episode. Inter-ICU transfers are considered part of the same continuous ICU episode. Deaths after discharge from the index ICU episode - whether on the ward or during any later ICU readmission within the same hospital admission - are not counted. This is considered from ICU admission onwards.
  - Hospital mortality: death occurring at any time during the index (initial) hospital admission, irrespective of location (ICU or ward within the same hospital) and including transfers to other hospitals within the same continuous admission. Deaths after discharge from the index hospital admission are not counted, even if the patient is readmitted later. This is considered from ICU admission onwards.
  - ICU length of stay: the number of calendar days from ICU admission to ICU discharge, including referral or transfer to other ICUs during the index ICU episode.
  - Hospital length of stay: the number of calendar days from ICU admission to hospital discharge, including transfers to other hospitals during the index hospital episode. Discharges or transfers to rehabilitation wards within the same hospital are not counted as hospital length of stay.

Efficacy endpoints:

- **Daily and cumulative sodium, chloride and glucose administration:**
    - Total daily amount of sodium, chloride and glucose (mmol or g per ICU day) from study fluids, non-study maintenance, replacement and resuscitation fluids, enteral and parenteral nutrition, and blood products (sodium/chloride content estimated from reference values). Excludes non-study fluid creep and oral intake. This is collected from ICU admission onwards until ICU discharge.
    - Cumulative sodium, chloride and glucose administration: running total of sodium, chloride, and glucose (mmol or g) from ICU admission onwards. Each day's administered amount is added to all previous ICU days. Same sources and exclusions as for daily administration.
- **Fluid balance:**
    - Daily fluid balance (mL per ICU day) is calculated as inputs minus outputs (see Table 2), excluding insensible losses.
    - Cumulative fluid balance: running total of daily net fluid balances (inputs minus outputs), excluding insensible losses. Each day's balance is added to that of all previous ICU days, allowing assessment at any time during the ICU stay.
    - From the moment RRT or IV loop diuretics are started, fluid balance data are analyzed separately from the patients without those interventions. Fluid balance assessments are discontinued from the ICU Day when the urinary catheter is removed, or bladder irrigation is initiated. This is collected from ICU admission onwards.

The necessary assessments for these endpoints are summarized in Table 2.

		Daily volume (mL)	Daily sodium (mmol)	Daily chloride (mmol)	Daily glucose (g)
Volume IN	Study-arm maintenance	x	x	x	x
	Study-arm fluid creep	x	x	x	x
	Non-study fluid creep	x			
	Non-study maintenance and replacement fluids	x	x	x	x
	Non-study resuscitation fluids (>500 mL/h)	x	x	x	x
	Nutrition (enteral and parenteral)	x	x	x	x
	Blood products	x	x	x	x

	Oral Intake	x			
Volume OUT	Urine	x	(SALADIN nested substudy)	(SALADIN nested substudy)	
	Net ultrafiltration (RRT)	x			
	Gastric fluid (excl. irrigation volumes)	x			
	Drain outputs	x			
	Diarrhea if precisely collected	x			

Table 2: Different components collected in the detailed assessment of fluid balance. Fluids are collected per ICU Day. The first ICU Day spans from admission to 8AM the following calendar day; subsequent ICU days run from 8 AM to 8 AM the following calendar day; the day of ICU discharge runs from 8AM to the moment of discharge.

4.3 Exploratory endpoint

- **Serum urea-to-creatinine ratio**

Difference in the serum urea-to-creatinine ratio measured every other ICU day from the first morning after randomization in patients not receiving renal replacement therapy.

Serum creatinine levels are routinely assessed, urea is not always standard-of-care, but will not lead to additional sampling as this will be performed together with routine morning blood sample.

Patients under renal replacement therapy are excluded from this exploratory endpoint, and urea assessments are discontinued from the moment RRT is started.

Exploratory endpoints in SALADIN substudy

- **Sodium and chloride balance**
    - Daily sodium and chloride balance (mmol per ICU day): net daily balance of sodium and chloride (mmol per ICU day), defined as study and non-study intake minus urinary output. Outputs are sodium and chloride content of all urine collected during the ICU day. Urine samples are performed until the ICU day on which bladder catheter removal, initiation of bladder irrigation, start of renal replacement therapy, or ICU discharge occurs, whichever comes first. This is collected from ICU admission onwards.
    - Cumulative sodium and chloride balance: running total of daily sodium and chloride balances. Each day's balance is added to that of all previous ICU days, allowing assessment at any time during the ICU stay. This is collected from ICU admission onwards.
- **Measured daily body weight**

- **Calculations of solute-free and electrolyte-free water clearance**
    - Solute-free water clearance (FWC) is calculated using urine flow rate (volume per ICU day), urine osmolality (assessed on study-specific urine collections) and plasma osmolality (assessed within standard of care). Reported as mL per ICU day.

      Formula: CH2O = V × (1 - Uosm/Posm),

      where V = urine flow rate, Uosm = urine osmolality, and Posm = plasma osmolality. Calculated daily during ICU stay in participants with 24-hour urine collections starting from ICU admission.
    - electrolyte-free water clearance (EFWC) is calculated using urine volume per day, urine sodium and potassium (both assessed on study-specific urine collections) and serum sodium (assessed within standard of care). Reported as mL per ICU day.

      Formula: CeH2O = V × (1 - (UNa + UK)/PNa))

      where V = urine flow rate, UNa = urine sodium concentration, UK = urine potassium concentration, and PNa = plasma sodium concentration.Calculated daily during ICU stay in participants with 24-hour urine collections starting from ICU admission.

- **Volume kinetics assessments**

Total volume and sodium intake, body weight and urine volume, sodium, chloride and potassium output will be correlated with measures of plasma dilution based on the standard-of-care measurements of hemoglobin, albumin and sodium, which are collected together with the timing of their assessment.

- **Bioelectrical Impedance Analysis**

Bio-Electrical Impedance Analysis (BIA) measurements will be performed using a touch i8 multi-frequency analyzer (Maltron International, Essex, UK) as per the manufacturer's instructions. Two electrodes will be placed on the hand and two on the forefoot at each side (in total 8 electrodes, 4 on the left and 4 on the right), and bioelectrical impedance will be measured at four frequencies (5, 50, 100, and 200 kHz), with STAR methodology allowing segmental analysis with the patient in a completely supine position. The multi-frequency approach allows differentiation between intracellular and extracellular as well as intravascular and extravascular compartments, providing critical insights into fluid status without exposure to radiation or other risks

Measurements will be conducted at least once every three ICU days starting from the day of randomization.

Parameters assessed:

- Total Body Water (TBW), Intracellular Water (ICW), and Extracellular Water (ECW) in liters and percentages, and ECW/ICW ratio
- Intravascular (IVF) and extravascular (EVF) fluid, and EVF/IVF ratio
- Volume excess (VE)
- Fat-Free Mass (FFM, kg and %), Fat-Free Mass Hydration (FFMH, %), and Fat Mass (FM, kg and %)

- Protein mass (kg), Mineral mass (kg), Bone mass (kg), Muscle mass (kg)
- Resting Metabolic Rate (RMR), Glycogen deposits (g)
- Phase angle and Malnutrition Index

5. SEQUENCE OF PLANNED ANALYSES

5.1 Interim analyses

We will conduct one interim-analysis when 320 participants have been followed up for 90-days.

The interim analyses will be performed by the unblinded statistician. In the interim analysis, a Mann-Whitney test of the primary outcome is performed with use of the conservative Haybittle-Peto boundary (two-sided significance level of 0.0027) which will allow the final analysis to be performed using an unchanged level of significance of 0.05.

In case the DSMB recommends to stop the trial, the DSMB will discuss and recommend on whether the final decision to stop the trial will be made after the analysis of all participants included at the time (including participants randomized after participant number 320) and whether a moratorium shall take place (setting the trial on hold) in the further inclusion of participants during these extra analyses. If further analyses of the participants included after 320 participants is recommended, the rules for finally recommending stopping of the trial should obey the Haybittle-Peto boundary. The DSMB will submit their recommendations to the TMG, which make the final decision regarding the continuing, pausing or stopping of the trial as described in the DSMB charter.

The trial will not be stopped for futility as an intervention effect less than a 16% relative risk reduction maybe clinically relevant. However, the DSMB can recommend pausing or stopping the trial if continued conduct of the trial clearly compromises participant safety.

5.2 Final analyses and reporting

All final planned analyses are conducted only after the last patient has completed the 90 days follow-up.

Any extra post-hoc exploratory analyses performed to provide support for planned analyses, but not mentioned in the SAP will be documented and reported in appendices and clearly marked as unplanned analyses in any publication.

6. STATISTICAL METHODS

6.1 Analysis principles

We will consider three analysis sets:

- Modified intention-to-treat population: All randomized patients with a signed written informed consent (see Informed Consent Procedure). In the unlikely event that a patient, upon regaining full capacity, requests complete deletion of their data, those data will be excluded from this analysis set.
- A per-protocol population are the patients from the modified intention-to-treat population without major protocol deviations. The exact conditions for the per-protocol population will be identified prior to data base lock.
- SALADIN substudy analysis set: the patients from the modified intention-to-treat population entered in the substudy and fulfilling the SALADIN in- and exclusion criteria

Two-sided 5% significance levels will be used to identify statistically significant results. All confidence intervals reported will be 95% confidence intervals.

6.2 Incomplete follow-up, missing data and outliers

6.2.1 Missing outcome data

The study coordinator(s) follow up on the missing data and records any reasons for missing data in patient file (medical record) and the eCRF (REDCap).

If less than 15% of data are missing for any primary or secondary outcome, a complete case analysis without imputation of missing values will be performed. If missing data are more than 15%, it will be assessed whether data are ‘missing completely at random’ (MCAR criterion) based on a rational assessment of the pattern of missing data and Little’s test in case of doubt. If it is concluded that data are not MCAR, multiple imputation using chained equations will be performed by creating 20 input datasets under the assumption that the data are ‘missing at random’.

In any multiple imputation, we will use all relevant outcomes and the stratification variables (site, invasive mechanical ventilation at randomization, surgical admission), age, sex, chronic kidney disease, hematologic or metastatic cancer, sepsis/septic shock, hypoxic respiratory failure (P/F ratio < 150) and severity of illness score (SMS-ICU). Multiple imputation will be performed separately in the two intervention groups before pooling the full dataset, and will serve as a sensitivity analysis to the unadjusted, non-imputed analysis.

6.2.2 Missing baseline covariates

Baseline covariates are assumed not to be missing.

6.2.3 Outliers

Outliers will be identified by examination of residual plots. Cases that stand out are further examined for possible influence on the results by comparing analyses with and without these cases. When there are discrepancies between both analyses, this will be reported.

6.3 Data transformations

Data transformations using natural logarithm, inverse or square root will be considered when model assumptions of normality and constant variance of the residuals are not met.

6.4 Multicenter study

The CRUSADERS study is a multicenter study with 4 sites: UZA, ZAS Palfijn, ZAS Cadix and ZAS Middelheim. In the sensitivity analysis of the primary outcome, Van Elteren test and regression model, there will be adjusted for the stratification variable site.

6.5 Multiple comparisons and multiplicity

No adjustments for multiplicity will be made, because outcomes and objectives are ordered by importance and results will be interpreted accordingly.

If in the mixed model time is significant as fixed effect, the different time points will be compared post-hoc to see where the differences lie. For this a Bonferroni-Holm multiple testing correction will be used.

6.6 Data management and analysis software

Data will be collected in REDCap.

All analyses will be performed in SAS version 9.4 or higher, or R version 4.3 or higher.

7. STATISTICAL ANALYSES

7.1 Patient characteristics and baseline comparisons

- Compare demographics like age, sex at birth, ethnicity, BMI between the NaCl-rich and the NaCl-poor arm. Means and standard deviation per group (or median, interquartile range as appropriate) will be reported. For ethnicity and sex, numbers and percentages are reported.
- Compare chronic comorbidities: HEFrEF, chronic kidney failure, chronic liver disease Child-Pugh B or C, diabetes mellitus type, hematologic or metastatic cancer between the NaCl-rich and the NaCl-poor arm (observed frequencies and percentages)
- Compare time from hospital admission to ICU admission (median, IQR)
- Compare time from ICU admission to randomization (median, IQR)

- Compare admission variables like source, type and main reason for admission between the NaCl-rich and the NaCl-poor arm. Sepsis or septic shock at admission will be reported per arm (observed frequencies and percentages).
- Compare severity score (SMS-ICU) and organ-failure score (SOFA) as well as individual markers of organ failure (vasopressor/inotrope use during first ICU day, P/F ratio at admission (hypoxic respiratory failure P/F ratio <150), use of invasive and non-invasive respiratory support during first ICU day, mechanical ventilation at randomization and renal replacement therapy during first ICU day) between the NaCl-rich and the NaCl-poor arm. For the categorical variables observed frequencies and percentages are reported, for the numeric variables mean (SD) or median (IQR) as appropriate. For the SMS-ICU the definition of Granholm et al. (2018) and for SOFA the definition of Vincent et al. (1996) *The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure* is used.
- Compare chronic treatment with loop diuretics between the NaCl-rich and the NaCl-poor arm (observed frequencies and percentages).
- Compare baseline sodium and chloride levels between the NaCl-rich and the NaCl-poor arm (mean (SD) or median (IQR) as appropriate). Baseline level is the final measurement obtained prior to randomization.
- A consort flow diagram will be produced to get an overview of the number of patients available at each stage: eligibility, randomisation, inclusion, discontinuation and follow-up.

7.2 Analysis of the primary endpoint

7.2.1 Primary analysis

The primary analysis will compare the primary outcome DAWOLS90 in the NaCl-rich and the NaCl-poor arm using a Mann Whitney test. This will be considered in the modified intention-to-treat population (see definition section 6.1)

7.2.2 Sensitivity analysis of primary endpoint

- Mann Whitney test of DAWOLS90 in per-protocol population (see definition section 6.1)
- The Van Elteren test adjusted for the stratification variables site, invasive mechanical ventilation at randomisation and surgical admission will be performed in the modified intention-to-treat and in the per-protocol population.
- A regression model that has a good fit (different models can be considered linear regression, general linear model with Poisson distribution or negative binomial distribution) where besides intervention (NaCl-rich or NaCl-poor) other possible confounders can be added (one by one or in combination). The following variables are considered:
    - age
    - sex
    - trial site
    - chronic kidney disease
    - hematologic or metastatic cancer
    - sepsis/septic shock
    - surgical admission
    - invasive mechanical ventilation at randomization
    - hypoxic respiratory failure (P/F ratio <150)
    - severity of illness score (SMS-ICU).

Multicollinearity will be checked when combining variables. Trial site will be considered as a random effect in this model.

- The same regression model but beside considering solely the intervention arm as being either NaCl-rich or NaCl-poor, amount of sodium due to study treatment will be added to the model as a continuous variable and interaction between this amount and intervention arm will be considered.
- These models will be considered in the modified intention-to-treat and in the per-protocol population.

7.2.3 Subgroup analysis of primary endpoint

- The following subgroup analyses are performed:
  - Patients with the following chronic comorbidities
    - Chronic liver disease Child-Pugh B or C
    - HEFrEF (LVEF ≤40%)
    - Chronic kidney disease (eGFR <60 ml/kg/1.73m²)
  - Patients with the following conditions at admission
    - Sepsis/septic shock (sepsis 3.0 definitions)
    - AKI creatinine stage 2 or higher (KDIGO classification)
    - Hypoxic respiratory failure (P/F ratio <150)

All these subgroups are binary categorical. To answer the question if the intervention effect is different in these subgroups a regression model (same as chosen previously) will be fitted using intervention, binary subgroup variable and the interaction between the intervention and the binary subgroup variable. If the coefficient with the interaction term is significantly different from zero, this variable will be seen as an effect modifier and the intervention effect in each of the subgroups with 95% confidence interval will be reported.

The subgroup analysis will be done in the modified intention-to-treat population.

7.3 Analysis of secondary endpoints

- For the binary secondary endpoints
  - occurrence of moderate hyponatremia (at least on one ICU day)
  - occurrence of severe hyponatremia
  - occurrence of moderate hypernatremia
  - occurrence of severe hypernatremia
  - occurrence of moderate hyperchloremia
  - occurrence of severe hyperchloremia
  - occurrence of hypoglycemia <70 mg/dL
  - development of new-onset AKI
  - development of new-onset RRT
  - development of new-onset need for mechanical ventilation
  - ICU mortality
  - hospital mortality

  A Fisher's exact test for unadjusted comparisons (or Chi-square test if assumptions allow) and a logistic regression model for adjusted comparisons will be considered using intervention, age, sex, trial site, mechanical ventilation at randomization, chronic kidney disease, sepsis/septic shock, hypoxic respiratory failure (P/F ratio <150), hematologic or metastatic cancer, surgical admission and severity of illness score (SMS-ICU). Confounders will added one by one to the model, combinations of variables can be

considered provided the number of events is sufficient to support them. Multicollinearity will be assessed when multiple variables are combined.

For all outcomes superiority of the intervention is expected. For moderate hyponatremia and severe hyponatremia non-inferiority of NaCl-poor compared to NaCl-rich arm will be evaluated by comparing the 95% confidence interval for the difference between the proportions of the intervention and control group with the non-inferiority margin of 5%. Non-inferiority is confirmed if the upper bound of the 95% confidence interval for proportion NaCl-poor arm – proportion NaCl-rich arm is below 5%.

- For hospital mortality a time-to-event analysis will be considered to compare the two arms. Start date: ICU admission, Event date: date of death from any cause occurring at any time during the index (initial) hospital admission, irrespective of location (ICU or ward within the same hospital) and including transfers to other hospitals within the same continuous admission. Deaths after discharge from the index hospital admission are not seen as an event, even if the patient is readmitted later. These patients are censored at hospital discharge date of the index hospital admission. If the patient is still alive and hospitalized on day 90 (i.e., remains in the index hospital episode), they are censored at day 90.
- The outcomes ICU and hospital length of stay will be modeled using a time-to-event analysis with death as a competing risk to compare the two arms.
  ICU length of stay: Start date: ICU admission, Event: ICU discharge, including referral or transfer to other ICUs following the same admission. In case of alive and in ICU at D90, censoring at D90.

  Hospital length of stay: Start date: ICU admission, Event: hospital discharge, including transfers to other hospitals following the hospital stay in which the patient was enrolled. Discharges or transfers to rehabilitation wards within the same hospital are not counted as hospital length of stay. In case of alive and in hospital at D90, censoring at D90.

- For the numeric variables:
  - days without renal replacement therapy at D90,
  - ventilator-free days at D90
  - days alive and out of hospital at D90
  - proportion of ICU days during which IV loop diuretics were administered
  - proportion of hyperglycemia over all glycemia measurements
  - cumulative fluid balance from ICU admission to the morning of the first ICU day on which an IV loop diuretic is prescribed

  a linear regression model (or models comparable to primary outcome if assumptions are not met) will be used. In these models there will be corrected for other variables like age, sex, trial site, invasive mechanical ventilation at randomization, chronic kidney disease, sepsis/septic shock, hypoxic respiratory failure (P/F ratio <150), hematologic or metastatic cancer, surgical admission and severity of illness score (SMS-ICU).

  For cumulative fluid balance (measured per day) time will be added to the model and subject as a random effect to correct for multiple measurements of the same subject.

  For hyperglycemia non-inferiority of NaCl-poor compared to NaCl-rich arm will be evaluated by comparing the 95% confidence interval for the difference between the proportions of the intervention and control group with the non-inferiority margin of 10%. Non-inferiority is confirmed if the upper bound of the 95% confidence interval for proportion NaCl-poor arm – proportion NaCl-rich arm is below 10%.

- For the time from randomization to first use of IV loop diuretics, a time-to-event analysis will be done comparing the NaCl-rich and NaCl-poor arm using a Kaplan-Meier plot and log rank test. When no IV loop diuretics are started during the initial ICU stay, patients are censored at time of initial ICU discharge. A Cox proportional hazards model can be considered if confounders need to be added.
- Mean daily glycemia for each ICU day are compared between the two arms using a linear mixed model.
- The efficacy endpoints
    - Daily sodium administration
    - Cumulative sodium administration
    - Daily chloride administration
    - Cumulative chloride administration
    - Daily glucose administration.
    - Cumulative glucose administration
    - Daily fluid balance on ICU days
    - Cumulative fluid balance on ICU days

  will be looked at graphically (taking into account diuretic use), described with descriptive statistics and if data allows modelled using linear mixed models (as they are measured repeatedly).
- The safety and efficacy endpoints will be considered in the modified intention-to-treat and the per-protocol population.

7.4 Analysis of safety endpoints

The following SARs:

- New-onset (starting > 48h after randomization) severe hyponatremia (<125 mmol/L) with seizures or profound lethargy unexplained by sedation or other disease.
- New-onset (starting > 48h after randomization) severe hypernatremia (>155 mmol/L) with seizures or profound lethargy unexplained by sedation or other disease.
- New-onset (starting >24h after randomization) **severe hypoglycemia (< 40 mg/dL)**
- New-onset **central pontine myelinolysis** (symptoms starting > 48h after randomization), confirmed on MRI within 90 days of admission
- New-onset (starting > 24h after randomization) **clinical refeeding syndrome**, defined as severe hypophospatemia < 1 mg/dL or < 0.32 mmol/L + hypokalemia < 3 mmol/L + clinical picture with symptoms such as fluid retention, edema, extreme muscle weakness, cardiac arrythmias, heart failure, respiratory failure, seizures or a combination of the above.
- New onset (starting > 24h after randomization) **Wernicke encephalopathy** defined by the triad of ophthalmopegia, ataxia and confusion.
- **Anaphylactic reactions presumed to be caused by the study treatment**, defined as urticaria and at least one of following: hemodynamic instability, increased airway resistance, stridor or bronchospasm treated with bronchodilators.

and SUSARs will be summarized per arm using descriptive statistics.

7.5 Exploratory analyses

- The exploratory endpoints CRUSADERS will be considered in the modified intention-to-treat population:
  - Serum urea-to-creatinine ratio (urea divided by creatinine)
- The exploratory endpoints SALADIN will only be considered in the SALADIN substudy analysis set:
  - Daily sodium and chloride balance
  - Measured daily body weight
  - Solute-free water clearance
  - Electrolyte-free water clearance
  - Volume kinetics assessments: Total volume and sodium intake, body weight and urine volume, sodium, chloride and potassium output will be correlated with measures of plasma dilution based on the standard-of-care measurements of hemoglobin, albumin and sodium, which are collected together with the timing of their assessment.

  - BIA, the parameters assessed are:
    - Total Body Water (TBW), Intracellular Water (ICW), and Extracellular Water (ECW) in liters and percentages, and ECW/ICW ratio
    - Intravascular (IVF) and extravascular (EVF) fluid, and EVF/IVF ratio
    - Volume excess (VE)
    - Fat-Free Mass (FFM, kg and %), Fat-Free Mass Hydration (FFMH, %), and Fat Mass (FM, kg and %)
    - Protein mass (kg), Mineral mass (kg), Bone mass (kg), Muscle mass (kg)
    - Resting Metabolic Rate (RMR), Glycogen deposits (g)
    - Phase angle and Malnutrition Index

    correlation of BIA data with fluid and electrolyte balance, measures of free water clearance, body weight, volume kinetics calculations and clinical outcomes in the SALADIN nested substudy

  will be looked at graphically (taking into account diuretic use), described with descriptive statistics and if data allows modelled using linear mixed models (as they are measured repeatedly). For the exploratory SALADIN endpoints the linear mixed models will also allow to study associations among the different endpoints.
- A sensitivity analysis can be considered on the DAWOLS90 outcome using the actual value of days alive without penalizing death.

8. REPORTING CONVENTIONS

For linear regression models and linear mixed effects models, coefficients, 95% confidence intervals and p-values will be reported. For logistic and Cox regression models respectively odds ratios and hazard ratios, together with 95% confidence intervals and p-values will be given.