Statistical analysis plan PUSH-AHF

Trial number	NL75163.042.20 NCT04606927
Title	Pragmatic Urinary Sodium-based treatment algoritHm in Acute Heart Failure PUSH-AHF
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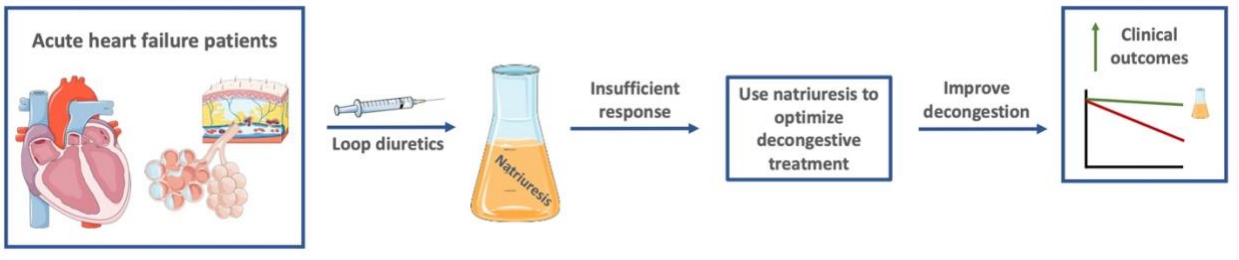

1. Document History

Version number	Version date	Author	Reason for change
Version 1.0	1.0	ter Maaten, Damman, Postmus	First version

2. Table of contents

3 List of abbreviations

EAC	Endpoint Adjudication Committee
HF	Heart Failure
ICH	International Conference on Harmonisation
ITT	Intention To Treat
NT-proBNP	N Terminal pro Blood Natriuretic Peptide
PUSH-AHF	Pragmatic Urinary Sodium-based treatment algoritHm in Acute Heart Failure
SAP	Statistical Analysis Plan
SD	Standard Deviation

4 Introduction

As per the International Conference on Harmonisation (ICH) E9 guidance, the purpose of this document is to provide a more technical and detailed description of the analysis described in the protocol. The statistical analysis plan (SAP) assumes familiarity with the trial protocol, which may be consulted for more background information on the study. R version 4.3.0 will be used for all analyses.

5 Study synopsis

The Pragmatic Urinary Sodium-based treatment algoritHm in Acute Heart Failure (PUSH-AHF) trial is a pragmatic, single-center, randomized, controlled, open-label study, comparing natriuresis guided therapy with standard of care in patients with acute heart failure requiring treatment with intravenous loop diuretics.

5.1 Study objectives and endpoints

5.1.1 Rationale

Administration of loop diuretics to achieve decongestion is the current cornerstone of therapy for acute heart failure. Unfortunately, there is a lack of evidence on how to guide diuretic treatment. Recently, urinary sodium, as a measure of diuretic response, has been proposed as a target for therapy. The hypothesis of this study is that natriuresis guided therapy in patients with acute heart failure will improve diuretic response, and reduce long term clinical outcome.

5.1.2 Primary endpoint

The dual-primary endpoint is defined as: a) total 24-hour natriuresis and b) first occurrence of all-cause mortality or adjudicated heart failure (HF) rehospitalization at 6 months after randomization.

Primary Endpoint:	**Outcome Measure:**
To determine whether a natriuresis guided diuretic strategy can improve natriuresis and clinical outcomes (all cause death, HF rehospitalization)	1) Total Natriuresis at 24 hours after randomization 2) Time to the first occurrence of any of the components of this composite: a. All cause Death b. (Adjudicated) HF Rehospitalization

5.1.3 Secondary endpoints in prespecified hierarchical order

Key secondary endpoints are:

- Total 48-hour natriuresis (from 0-48 hours)
- Total diuresis at 24-hours (from 0-24 hours)
- Total diuresis at 48 hour (from 0-48 hours)
- Length of hospital stay from baseline to discharge
- HF rehospitalization: time to first event, total number of HF rehospitalizations (recurrent events)
- All-Cause Mortality

Secondary Endpoints:	**Outcome Measure:**
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total natriuresis after 48 hours	Total natriuresis (mmol) from randomization to 48 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total diuresis after 24 hours	Total diuresis (mL) from randomization to 24 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total diuresis after 48 hours	Total diuresis (mL) from randomization to 48 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on length of stay of the index hospitalization	Number of days in hospital for index HF admission
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total number of recurrent HF hospitalizations	Total number of (first and recurrent) HF hospitalizations
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on all-cause mortality	Time to death from any cause
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on percentage change in NT-proBNP at 48 hours from baseline	Percentage change in NT-proBNP at 48 from baseline
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on percentage change in NT-proBNP at 72 hours from baseline	Percentage change in NT-proBNP at 72 from baseline

5.1.4 Safety endpoints

Safety endpoints include:

- SAE's
- Renal Safety Events
    - Doubling of serum creatinine from baseline to 24 hours
    - Doubling of serum creatinine from baseline to 48 hours
- Pre-specified adverse events:
    - Worsening heart failure during hospitalization, defined as
        - Addition or starting of inotropes or vasopressors
        - Mechanical ventilation
        - Palliative care due to progressive heart failure
        - Any intervention/treatment for heart failure that leads to prolonged hospitalization
    - True worsening renal function, defined as
        - Doubling of creatinine from baseline to 48 or 72 hours without evidence of decongestion, or urine production < 10 cc / hour despite adequate dosing of loop diuretics

Safety Endpoints:	**Outcome Measure:**
To evaluate the safety and tolerability of a natriuresis guided diuretic strategy versus standard of care	1) Serious Averse Events (SAEs) 2) Renal Safety Endpoint: a. Doubling of serum creatinine from baseline to 24 or 48 hours 3) Pre-specified adverse events: a. Worsening heart failure b. True worsening renal function

5.1.5 Exploratory Endpoints

- Total 72 hour natriuresis (from 0-72 hours)
- Total 72 hour diuresis (from 0-72 hours)
- Net negative fluid balance at 6, 12, 24, 48 and 72 hours
- Incidence of Hypo/hyperkalemia in the first 72 hours.

Exploratory Endpoints:	Outcome Measure:
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total natriuresis after 72 hours	Total natriuresis (mmol) from randomization to 72 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on total diuresis after 72 hours	Total diuresis (mL) from randomization to 72 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on net negative fluid balance at 6, 12, 24, 48 and 72 hours	Fluid balance (Fluid Intake minus Diuresis) for each time interval after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on change in body weight at 24, 48 and 72 hours	Weight at baseline, 24, 48 and 72 hours
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on diuretic response at 24, 48 and 72 hours	Weight change per 1 mg Bumetanide (40 mg furosemide) at baseline, 24, 48 and 72 hours
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on change in NTproBNP	Percentage change in baseline NTproBNP at 24, 48 and 72 hours after randomization
To compare the effect of a natriuresis guided diuretic strategy versus standard of care on changes in serum potassium levels and incidence of hypokalemia (< 3.5 mmol/L) or hyperkalemia (> 5.5 mmol/L)	Change in serum potassium levels up to 72 hours and the incidence of hypo- and hyperkalemia

5.2 Definitions

5.2.1 End of study date

The study will be finished when the last follow-up visit of the last enrolled patient (last patient, last visit) is completed. This will be 180±10 days after inclusion of the last patient.

5.2.2 Withdrawal of consent

Withdrawal of consent means withdrawal from the study and should only occur if the patient does not agree to any further assessment at all. No data after date of withdrawal of consent should be collected. Data collected on or prior to date of withdrawal of consent will be included in the analyses.

5.2.3 Cross-over from treatment allocation group

Cross-over from treatment allocation group does not mean exclusion from the study or withdrawal of consent. Optimally, in these patients the follow-up visit should be completed per protocol. Data from these patients will be included in the intention to treat analyses according to their allocated treatment group. Events are included irrespective of the timing of the event in relation to the moment of cross-over.

5.2.4 Vital status

Known vital status at the end of the study is defined known whether the patient is dead or alive at the follow-up visit.

5.2.5 Lost to follow-up

The term lost to follow-up will be limited to patients with unknown vital status at the end of the study as defined in section 2.2.4.

5.3 Study design

This is a pragmatic, randomized, controlled, open label study in patients presenting with acute heart failure requiring intravenous loop diuretics. In total 310 patients will be randomized at the start of intravenous treatment (inhospital) to standard of care of natriuresis guided treatment. In the natriuresis guided treatment arm, decongestive treatment will be adjusted based on the spot urine sodium value assessed at set time points. Patients will be followed for the duration of the hospitalization. Adverse clinical events, including all-cause mortality and heart failure rehospitalization will be assessed by a telephone call after 6 months.

5.3.1 Randomization

Subjects will be randomized 1:1 to natriuresis guided therapy or standard of care. Randomization will be executed using an internal random number generator in the Electronic Health Record (EHR, EPIC, Verona, WI, USA). Each patient will be randomly assigned to either treatment arm when a patient's chart is opened by an eligible physician. This allocation is maintained as fixed variable in the EHR, ensuring randomization is only carried out onceWhen treatment with intravenous loop diuretic therapy is started, based on this randomization number, the Epic electronic health record automatically chooses the correct treatment orders (Orderset) and diagnostic tests based on the randomization arm.

5.3.2 Blinding

The study has an open label design, which means both patient and physician will know which randomized treatment the patient has been allocated to. However, to prevent contamination and cross-over between treatment arms, physicians will be blinded entirely to all urinary

sodium measurements (timed collections as well as spot urinary sodium) in the standard of care arm. These measurements will however be carried out at the local laboratory (which ensures the same measurement of our primary endpoint of 24 hour natriuresis), but results will be withheld at the laboratory department and only after the 6 month follow up period has passed will be send to the EHR. Additional alerts are in place to ensure that physicians are instructed not to order (unblinded) urinary sodium assessment in the standard of care arm, unless there is an important medical reason.

5.3.3 Number of patients

Based on a previous observational study in acute heart failure patients, the mean 24 hours sodium excretion was 398±246 mmol. In this population, not 2 hours, but 6 hours measurements were available, and in these patients, 36% of patients had an insufficient response, defined as urinary sodium <90 mmol or urine output <900 mL at 6 hours. Assuming a 40% improvement in these 36% of patients, and a conservative 15% improvement in the remaining patients in their 24-hours urinary sodium excretion because of closer monitoring compared with standard of care, this will assume an overall 24% improvement in 24-hours urinary sodium excretion (0.36*1.40 + 0.64*1.15). Therefore, to obtain a power of at least 80% at a two-sided significance level of 0.025 (Bonferroni correction for a dual-primary endpoint), we calculated a sample size of 125 patients in each group would be sufficient for the primary endpoint of 24-hours natriuresis. To prevent being underpowered due to drop-out or missing data, which is expected to be higher than average in this pragmatic study design and given the delicate nature of urinary collections, we will increase enrolment by 10% therefore requiring 140 patients per group, and enrolling a total of 280 patients.
Based on this sample size, we will have 81% power with a two-sided significance level of 0.025 to detect a hazard ratio of 0.49 for the other dual-primary endpoint of all-cause mortality and heart failure rehopitalization at 6 months (a reduction in events from 38% to 21%). However also accounting for 10% missing follow-up data, we will increase the total number of patients to 310 (155 patients per group). After closing of the database, a CONSORT diagram will be produced for transparent status of the subject reporting.

6 Analyses sets

There will be two treatment regimens in this trial, namely natriuresis guided or standard of care. The efficacy analyses will follow the intention to treat (ITT) principle in assigning patients to treatment groups, i.e. patients will be analyzed as randomized. Safety analyses will also assign patients to the treatment group as randomized.

- Full analysis set: all patients who have been randomized will be included in the full analysis set. This is the primary analysis set for the primary and secondary outcome variables. Following the intention-to-treat principle, patients are analyzed according to the treatment group they have been assigned to at randomization.
- Per protocol set: this is a subset of the full analysis set which consists of all randomized patients in the full analysis set that remained in their allocated treatment group for at least the first 24-hours and had no major protocol deviations. Major protocol deviations are those affecting the primary endpoint analyses.
- Safety analysis set: all randomized patients who were at least two hours in their allocated treatment group. Patients will be analyzed according to treatment group received.

All protocol deviations will be evaluated before database lock to determine whether patients can be included in the above described analyses sets. The most important protocol deviations listed below will be summarized by randomized treatment group:

- Patients who were randomized but did not meet inclusion and exclusion criteria
- Patients who received treatment based on the other allocation group (i.e. crossed over)
- Patients who either received insufficient amount of diuretics (either dose or combination diuretic therapy) or too much diuretics (dose or combination diuretic therapy) according to protocol
- Accidental unblinding of urinary sodium values in standard of care arm

7 Endpoint variables

7.1 Dual-primary endpoint

The primary endpoint of the PUSH-AHF trial consists of two distinct dual-primary endpoints, namely (i) total 24-h natriuresis and (ii) the first occurrence of the combined endpoint of all-cause mortality or HF rehospitalization at 6 months. The second part of the endpoint (time from randomization to the first occurrence of HF rehospitalization) will be adjudicated by the endpoint adjudication committee (EAC). Patients who did not have an event will be censored at the date of follow-up visit. Heart failure rehospitalization is defined as a hospital admission with signs and symptoms of heart failure requiring treatment. Members of the EAC are provided a complete, blinded, endpoint package for each rehospitalization. The package is assessed by the EAC members. In case of discrepancy between the evaluation of EAC members regarding the same event, the case is further discussed with the aim of developing a consensus. If the committee is unable to reach a consensus, the endpoint will be established by the EAC chair.

7.2 Secondary endpoints

The secondary endpoints are included in a hierarchical testing sequence following the primary endpoint, and will be tested if one of the dual-primary endpoints is statistically significant (at p-value 0.025). For the secondary outcomes, a two-sided P-value of 0.05 will be used if both primary endpoints are met. If only one primary endpoint will be met, a two-sided p-value of 0.025 will be used. Length of index hospitalization is calculated as the date of discharge from the hospital minus the date of screening plus 1. Patients still in the hospital at day 60 will be censored at day 60. If patients die during the index hospitalization, the maximum length of stay (60 days +1) will be assigned.

8 Statistical analysis

8.1 Closed Testing Procedure

The statistical analysis will use a pre-specified closed testing procedure, including a pre-specified hierarchical ordering of primary and secondary analysis. For the primary analysis, the type I error will be controlled at the two-sided 0.025 level, please see for more information 8.2. For the secondary endpoint, the type I error will be controlled at the two-sided 0.05 level. Statistical significance will continue in pre-specified order until an endpoint is rejected at two-sided 0.05 level.

8.2 Analysis of the primary endpoint

The primary endpoint will be assessed in the intention to treat (ITT) population. The dual-primary endpoint will be total 24-hour natriuresis and first occurrence of all-cause mortality or heart failure rehospitalisation at 6 months. Following the Bonferroni correction used for the sample size calculation, the study will be deemed positive if one or both of the components of the dual-primary endpoint are positive (*P-value*<0.025).

24-hour natriuresis will be calculated and presented as mean +/- SD if normally distributed, or median (25^{th} and 75^{th} percentile) in the case of non-normal distribution. The between group difference will be tested using t-test if normally distributed, or Wilcoxon rank sum test if non-normally distributed. The effect of natriuresis guided treatment on long-term outcomes will be assessed using Cox regression (assuming proportional hazard assumption is met) for the between treatment difference. Kaplan-Meier estimates will be calculated and plotted.

For the presentation of baseline characteristics in both treatment arms, continuous variables will be presented as mean +/- SD, non-normally distributed variables as median (25^{th} – 75^{th} percentile), and categorical values as count (percentages).

8.3 Secondary endpoint(s)

Secondary outcomes will be tested in the hierarchial testing scheme in the prespecified order of the endpoints as mentioned in 5.1.2 and 5.1.3. If both primary outcomes are rejected at two-sided p-value 0.025, no P-values will be calculated for the secondary outcomes.
Changes in biomarkers, natriuresis at subsequent time points, and length of hospital stay will be assessed using t-test for normal distributed variables, and Wilcoxon rank sum test if non-normally distributed.

8.4 Subgroup Analysis

For explanatory purposes subgroup analysis for the primary endpoint will be conducted, to assess whether the treatment effect is modified by different baseline characteristics. The table below gives an overview of the subgroups that will be investigated and how they will be created:

Characteristic	**Subgroup**
Age (years)	≤ median, > median
Sex	Male, Female
Left ventricular ejection fraction (if known)	≤ 40%, > 40%
Baseline NT-proBNP (ng/L)	≤ median, > median
Baseline estimated Glomerular Filtration Rate	≤ median, > median
Cause of heart failure	Ischemic, non Ischemic
Outpatient dose of loop diuretic	≤ median, > median
Hyponatremia (mmol/L)	≤ 135, > 135
Hypokalemia (mmol/L)	≤ 3.5, > 3.5
Atrial Fibrillation	Yes, No
SGLT2 inhibitor use at baseline	Yes, No

Between group differences in the second part of the dual-primary endpoint will be tested with use of a Cox proportional hazards model with the inclusion of an interaction term.

8.5 Other study parameters

Adverse event rates, including doubling of serum creatinine at 24 or 48 hours, serious adverse events, as well as worsening heart failure will be analyzed using Fisher's exact tests, and presented as counts (percentages). To study differences in repeated assessments between treatment groups, such as natriuresis during hospitalization, repeated measures linear mixed effect modeling will be used.

8.6 Missing data

The proposed statistical approach assumed missing data mechanism to be missing at random. If the level of missing data for the primary outcome in the full analysis set exceed 10%, a sensitivity analysis to assess the robustness of the analysis of the primary outcome by means of multiple imputation technique will be performed for the primary endpoint.

8.7 Other sensitivity analysis

This trial has been initiated during the COVID-19 pandemic, therefore no sensitivity analysis for the effect of COVID on heart failure hospitalization was deemed necessary.

8.8 Interim analysis (if applicable)

Not applicable

8.9 Pre-specified sub-analyses

In addition to the primary, secondary and exploratory endpoints and analyses as indicated in this SAP we prespecify the following analyses to be conducted after the main study results have been published.

8.9.1 Clinical Outcome

In this analysis, focus will be on the effect of natriuresis guided therapy on hard clinical endpoints, during and after hospitalization. This includes (but not restricted to) all cause mortality, cardiovascular and non-cardiovascular mortality and (repeated) hospitalizations, including heart failure hospitalization.

8.9.2 Congestion Status

In this analysis, focus will be on signs and symptoms of congestion. Association between treatment allocation, changes in congestion status, including biomarkers and associated outcome will be assessed.

8.9.3 Renal Function

In this analysis, the association between randomized treatment allocation and (changes) in renal function will be evaluated, including relationship with other parameters such as outcome.

8.9.4 HF Phenotype (HFrEF, HFmrEF, HFpEF)

In this analysis, the association between randomized treatment allocation and outcomes will be evaluated in different phenotypes of HF and the interaction between phenotype and treatment effect will be studied.

8.9.5 Win Ratio

In this analysis, the effect of randomized treatment on a Win-Ratio will be evaluated. One of the proposed win-ratio's will be a composite hierarchical investigated endpoint, including all cause death, HF rehospitalization and 24h natriuresis.

8.9.6 Home Diuretic Use

In this analysis, considering the association between diuretic responsiveness and home diuretic use and dose, the association between randomized treatment allocation and home diuretic use with outcomes will be evaluated

8.9.7 Pharmacodynamic substudy

In a small subgroup of patients included in the main trial, sequential urinary (and serum) samples have been obtained (via additional informed consent). In this subgroup of patients, the short-term effect of intravenous diuretic bolus treatment on diuresis, natriuresis will be evaluated.

8.9.8 Renal Ultrasound substudy

In a subgroup of patients included in the main trial, sequential renal ultrasounds have been executed (via additional informed consent). The main focus of this substudy is to evaluate the effect of diuretic treatment and randomized treatment allocation on echographic markers of (renal) congestion

8.9.9 Combined Analysis with ESCALATE

In this pre-specified analysis, we aim to combine data from PUSH-AHF with data from ESCALATE (NCT04481919). ESCALATE is different randomized, blinded controlled trial where a diuretic strategy, based on natriuresis, is tested against standard of care. We aim to include patient level data to investigate the effect of natriuresis guided treatment on heart failure outcomes, including natriuresis, diuresis, congestion parameters and clinical outcome. We have been into contact with the principle investigators of ESCALATE, who agree on this pre-specified analysis of both trials.