Appendix B: Statistical Analysis Plan

Diuretic Treatment in Acute Heart Failure with Volume Overload Guided by Serial Spot Urine Sodium Assessment (DECONGEST) Study

Version 1.0

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1. Study Objectives and Endpoints

1.1. Primary objective and outcome measure

Primary objective	Primary endpoint
To determine whether an intensive diuretic regimen focused on early combination therapy, based on serial post-diuretic spot urine sodium concentration (UNa^+) assessments, is safe and leads to faster and more effective decongestion	Hierarchical composite endpoint of: 1. survival at 30 days 2. days alive out of hospital or care facility up to 30 days 3. relative decrease in N-terminal of pro-hormone B-type natriuretic peptide (NTproBNP) levels from baseline to day 30

Definition of the primary endpoint:

Vital status is assessed at 30 days after randomization. Deceased subjects get the lowest rank for the analysis. Surviving subjects are subsequently compared for the total number of days from randomization to day 30 that they are alive without hospital admission or admission to a care facility to declare a winner. An admission day is defined by an overnight stay within the hospital (including the emergency department) or care facility. In surviving subjects, tied for the number of days without hospital or care facility admission, the relative decrease in NTproBNP from baseline levels to day 30 (rounded to the nearest percentage) is assessed and compared to declare a winner (the one with the greatest decrease, if the difference between them is at least 5%).

1.2. Secondary objectives and outcome measures

Secondary objective	Secondary endpoints
To further explore whether the studied intervention leads to more effective decongestion	Relative NTproBNP decrease from baseline to day 30 [%]
	Relative carboxyhydrate antigen 125 (CA-125) decrease from baseline to day 30 [%]
	Successful decongestion, defined as no more than trace oedema, absence of jugular venous distension and no rales upon the moment of transition from intravenous diuretics to oral maintenance therapy [%]
	Length of intravenous diuretic therapy [days]

To further explore whether the studied intervention leads to faster decongestion	Length of the index hospital admission [days]
To assess whether the studied intervention is associated with more symptomatic relief of congestion	5-point Likert scale for overall well-being assessed upon the transition from intravenous diuretics to oral maintenance therapy compared to baseline (much improved/slightly improved/neutral/slightly worse/much worse)
To assess whether the studied intervention is safe without significant renal adverse events	Doubling of the serum creatinine or plasma cystatin C compared to baseline with an absolute value >2 mg/dL or >2 mg/L, respectively, or the need for ultrafiltration and/or renal replacement therapy at any time during the index hospital admission
To assess whether the studied intervention is safe without significant hemodynamic adverse events	Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg or use of vasopressors and/or inotropes at any time during the index hospital admission
To assess the impact of the studied intervention on clinical outcomes	Death, non-elective readmission or non-elective medical contact after discharge within the 30-day follow-up period
	Death or non-elective readmission after discharge within the 30-day follow-up period

Definitions of the secondary endpoints

- NTproBNP is measured with the Elecsys proBNP II test on the cobas® e801 immunoassay analyser (Roche Diagnostics).
- CA-125 is measured with the Elecsys CA 125 II test on the cobas® e801 immunoassay analyser (Roche Diagnostics).
- Length of intravenous diuretic therapy is defined as the date of transition from intravenous diuretics to oral maintenance therapy minus the date of randomization +1.
- Length of the index hospital admission is defined as the date of discharge from the hospital minus the date of randomization +1.
- Cystatin C is measured with the Tina-quant Cystatin C Gen.2 test on the cobas® c 501 (Roche Diagnostics).
- Non-elective readmission is defined as any unforeseen hospital admission with at least 1 overnight stay (including the emergency department. Hospital admissions for planned procedures are not counted in this respect.

- Non-elective medical contacts are any unforeseen contacts of a patient with a medical caregiver at the patient's initiative for any health problem.

1.3. Exploratory outcome measures

Outcome Measure	Measure Description
Overall well-being at discharge	5-point Likert scale for overall well-being upon the moment of hospital discharge from the index hospitalisation compared to baseline (5: much improved/4: slightly improved/3: neutral/2: slightly worse/1: much worse).
Overall well-being after 30 days	5-point Likert scale for overall well-being at 30 days after randomisation compared to baseline (5: much improved/4: slightly improved/3: neutral/2: slightly worse/1: much worse).
Edema score after decongestion	Oedema score (1+: trace; 2+: ankle; 3+: knee; 4+: above knee) upon the moment of transition from intravenous diuretics to oral diuretic therapy.
Edema score at discharge	Oedema score (1+: trace; 2+: ankle; 3+: knee; 4+: above knee) upon the moment of hospital discharge from the index hospitalisation.
Edema score after 30 days	Oedema score (1+: trace; 2+: ankle; 3+: knee; 4+: above knee) at the 30-day follow-up visit.
Weight change with decongestion	Weight change [kg] from baseline to the moment of transition from intravenous diuretics to oral diuretic therapy.
NTproBNP change after decongestion	Relative NT-proBNP change [%] from baseline to the moment of transition from intravenous diuretics to oral diuretic therapy.
CA-125 change after decongestion	Relative CA-125 [%] change from baseline to the moment of transition from intravenous diuretics to oral diuretic therapy.
Change in estimated glomerular filtration rate (eGFR) after 30 days (serum creatinine-based)	Change in eGFR from baseline to the 30-day follow-up visit [mL/min/1.73m²] with eGFR calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula with serum creatinine.
Change in eGFR after 30 days (plasma cystatin C-based)	Change in eGFR from baseline to the 30-day follow-up visit [mL/min/1.73m²] with eGFR calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula with plasma cystatin C.
Change in eGFR after 30 days (serum creatinine/plasma cystatin C-based)	Change in eGFR from baseline to the 30-day follow-up visit [mL/min/1.73m²] with eGFR calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula with serum creatinine and plasma cystatin C.
Hyperkalemia	Hyperkalemia with serum potassium levels >5.5 mmol/L at any time during the study period.
Severe hyperkalemia	Severe hyperkalemia with serum potassium levels >6.5 mmol/L at any time during the study period.
Hypokalemia	Hypokalemia with serum potassium levels <3.5 mmol/L at any time during the study period.
Hyponatremia	Hyponatremia with serum sodium levels <135 mmol/L at any time during the study period.
Severe hyponatremia	Severe hyponatremia with serum sodium levels <125 mmol/L at any time during the study period.

Hypernatremia	Hypernatremia with serum sodium levels >145 mmol/L at any time during the study period.
Severe metabolic acidosis	Severe metabolic acidosis with serum bicarbonate levels <20 mmol/L at any time during the study period.
E/e' after decongestion	Averaged medial/lateral E/e' ratio on transthoracic echocardiography upon the moment of transition from intravenous diuretics to oral diuretic therapy.
E/e' at discharge	Averaged medial/lateral E/e' ratio on transthoracic echocardiography upon the moment of hospital discharge from the index hospitalisation.
E/e' after 30 days	Averaged medial/lateral E/e' ratio on transthoracic echocardiography at the 30-day follow-up visit.
Peak left atrial longitudinal strain after decongestion	Peak left atrial longitudinal strain on transthoracic echocardiography upon the moment of transition from intravenous diuretics to oral diuretic therapy.
Peak left atrial longitudinal strain at discharge	Peak left atrial longitudinal strain on transthoracic echocardiography upon the moment of hospital discharge from the index hospitalisation.
Peak left atrial longitudinal strain after 30 days	Peak left atrial longitudinal strain on transthoracic echocardiography at the 30-day follow-up visit.
Tricuspid plane annular excursion over right ventricular systolic pressure (TAPSE/RVSP) ratio after decongestion	TAPSE/RVSP ratio on transthoracic echocardiography upon the moment of transition from intravenous diuretics to oral diuretic therapy.
TAPSE/RVSP ratio at discharge	TAPSE/RVSP ratio on transthoracic echocardiography upon the moment of hospital discharge from the index hospitalisation.
TAPSE/RVSP ratio after 30 days	TAPSE/RVSP ratio on transthoracic echocardiography at the 30-day follow-up visit.
B-lines after decongestion	Number of B-lines on lung ultrasound (8 thoracic sites) upon the moment of transition from intravenous diuretics to oral diuretic therapy.
B-lines at discharge	Number of B-lines on lung ultrasound (8 thoracic sites) upon the moment of hospital discharge from the index hospitalisation.
B-lines after 30 days	Number of B-lines on lung ultrasound (8 thoracic sites) at the 30-day follow-up visit.
VExUS score after decongestion	VExUS score for venous Doppler measurements (0: inferior vena cava diameter <2 cm; 1: inferior vena cava diameter ≥2 cm and normal Doppler measurements; 2: inferior vena cava diameter ≥2 cm and at least 1 severely abnormal* Doppler pattern in the Vv. hepaticae, V. portae or Vv. intrarenalis; 3: inferior vena cava diameter ≥2 cm and at least 2 severely abnormal* Doppler patterns in the Vv. hepaticae, V. portae or Vv. intrarenalis) upon the moment of transition from intravenous diuretics to oral diuretic therapy.
VExUS score at discharge	VExUS score for venous Doppler measurements upon the moment of hospital discharge from the index hospitalisation.
VExUS score after 30 days	VExUS score for venous Doppler measurements at the 30-day follow-up visit.

*The following Doppler patterns are considered severely abnormal: Vv. Hepaticae: systolic flow reversal; V. Portae: >50% pulsatility; Vv. Intrarenalis: monophasic diastolic flow

2. Randomization & Allocation Concealment

The DECONGEST study is a randomized clinical trial with 2 treatment arms. Patients are randomly assigned in a 1:1 ratio to standard of care or the care bundle that comprises the DECONGEST study intervention. Randomization is performed through an online, protected website, using the Castor Electronic Data Capture system (Castor, Amsterdam, the Netherlands) after confirming eligibility. Block randomization is performed, stratified by study centre and left ventricular ejection fraction (<50% versus ≥50%), with variable blocks of 2 or 4 subjects for allocation concealment.

3. Sample size calculation

According to a sample size simulation, 96 patients need to be enrolled and analysed in the DECONGEST study, given a 2-sided significance level (α) of 0.05, to yield a statistical power (1-β) equal to 90%. To account for a potential drop-out and the impact of correlation between outcomes, the targeted study sample size was set at 104 subjects.

Based on the results of the Acetazolamide in Decompensated Heart Failure with Volume Overload (ADVOR) trial, which recruited a similar population in a comparable setting, the anticipated 30-day mortality for DECONGEST was estimated at 5%, with no differences among the treatment arms.

To anticipate the true effect size, different components of the DECONGEST intervention were considered. First, the upfront use of acetazolamide in the ADVOR trial was associated with a 1-day reduction in the length of the index hospitalization. Second, a similar effect was seen with systematic urine sodium concentration assessment in the Pragmatic Urinary

Sodium-based Treatment Algorithm in Acute Heart Failure (PUSH-AHF) trial. As the use of acetazolamide was unneglectable in the PUSH-AHF trial, we anticipated an additive effect of 2 days (IQR: 4 days) decrease in length of the index hospitalization, combining both treatment. Several other parts of the DECONGEST intervention bundle justify a further additive effect: (1) the low-threshold use of chlorthalidone and canreonate would be expected to have an independent effect to reduce index hospitalization length; (2) the loop diuretic dosing according to eGFR could reduce the number of patients with loop diuretic resistance due to underdosing and might speed up the decongestion process. Additionally, the more intensive diuretic schedule would result in lower NTproBNP levels in the intervention group, reducing the number of ties compared to ADVOR and turning them into wins for the intervention group. Based on the ADVOR trial a difference of 25% (with standard deviation of 22%) is anticipated in the relative decrease in NTproBNP. A difference of at least 5% between two subjects is set as a threshold to declare a winner. If the difference in NTproBNP decrease is less than 5% between two subjects, they're considered a tie.

4. Statistical analysis

4.1. General principles

This statistical analysis plan dated May 15, 2024, is based on the statistical information documented in the finally approved study protocol version 1.3 dated April 15, 2024. The scope of this statistical analysis plan is to outline the statistical tools used for the primary and secondary objectives of the DECONGEST study following the procedures documented in the study protocol version 1.3 in both participating centres. Prior to the statistical analysis, the collected study data will be cleaned by the data manager of the University Hospital Brussels (Dr. Bram Roosens). All statistical hypotheses are 2-sided and a 5% significance level will be used. If the primary endpoint is statistically significant, secondary endpoints are subsequently tested in a predefined hierarchical order to

preserve alfa spending without further correction for multiple testing. If the primary endpoint or any of the secondary study endpoints is non-significant, all following endpoints are considered explorative and no formal statistical testing will be reported in the main paper for these endpoints. Trial results will be reported according to the CONSORT statement on reporting randomized controlled trials. For the analysis of the primary outcome and each non-exploratory secondary outcome the following information will be presented:

- the number of patients included in each analysis, by treatment arm
- a summary statistic of the outcome by treatment arm
- the estimated treatment effect
- a 95% confidence interval for the estimated treatment effect
- A two-sided p-value for all analyses, a significance level of 5% will be used.

All statistical analysis will be done with SAS (SAS institute, Cary, NC, United States) and R.

4.2. Interim analysis

No interim analysis is planned regarding the DECONGEST study.

4.3. Multiplicity adjustment

There will be no correction for multiplicity of testing as there is only one primary endpoint. Secondary endpoint are tested in a hierarchical fashion to avoid type I error inflation.

4.4. Independent statistician

Once the database is cleaned, the dataset will be locked and transferred to an independent academic statistician (Johan Verbeeck –Hasselt University) for analysis according to this statistical analysis plan.

4.5. Datasets to be analysed

For all endpoints, the statistical analysis will be based on a modified intention-to-treat analysis set, including all randomized patients that received at least one dose of diuretics according to the study protocol. Patients will be analysed according to the treatment group they were allocated to according to the electronic randomization system (irrespective of the actual treatment received).

4.6. Subject disposition

After closing of the database, a CONSORT diagram will be produced for transparent status of the subject reporting.

4.7. Protocol violations and deviations

The important protocol deviations listed below will be summarised by randomized treatment group:

- Patients who were randomized but did not meet inclusion and exclusion criteria
- Patients in the standard of care arm in whom UNa^+ assessment was performed and patients with missing UNa^+ assessments in the intervention arm
- Patients in the intervention arm who received the wrong diuretic treatment according to the protocol at any time during the study

As the primary analysis is a modified intention-to-treat analysis, protocol deviation will not imply exclusion from the primary analysis.

4.8. Concomitant therapies

The frequency of baseline medications will be shown for the modified intention-to-treat analysis set per treatment group. Counts and percentages will be presented, grouping the baseline medication per drug classes.

4.9. Baseline characteristics

To describe the study population, characteristics of all the patients in the modified intention-to-treat analysis set per treatment group will be presented. Numbers (%), means ± standard deviation (SD) and medians (interquartile range) will be given for each treatment group, as appropriate.

4.10. Analysis of the primary outcome

The primary statistical analysis in the DECONGEST study will be a modified intention-to-treat (see section 4.5). The generalized pairwise comparison method is used to calculate a net treatment benefit for the hierarchical composite primary endpoint (see section 1.1). Every patient from the standard-of-care group will be pair-wise compared with each patient from the DECONGEST intervention group to declare a winner or tie. The following criteria are sequentially assessed to declare a winner or a tie:

1. Any subject surviving until 30 days after randomization will win from a subject who died during the same timeframe. If both subjects did not survive until the 30-day follow-up, there is a tie.

2. In a pair of subjects, both surviving up till the 30-day follow-up moment, the number of days in-hospital or in a care facility are counted (as in section 1.1.). The subject with the highest number of days alive and out of hospital or care facility during the 30-day follow-up window will be declared the winner.

3. In a pair of subjects, both surviving up till the 30-day follow-up moment and with the same number of days alive and out of hospital or care facility, the subject with the greatest relative reduction in NTproBNP from baseline, rounded to the closest percentage will be declared the winner, if the difference between 2 subjects is at least 5%. In case of relative NTproBNP reduction smaller than 5%, there is a tie.

To calculate the net treatment benefit in the DECONGEST study, essentially, each individual in the intervention group is compared against each individual in the control group and the resulting pair is assigned a score of +1, -1, or 0, depending on whether the pair is a win, a loss, or a tie, respectively. The net treatment benefit is subsequently calculated as the average of scores for all possible pairs and reported with a 95% confidence interval and the associated p-value. To aid the interpretation of the results, the proportions of wins and losses for each outcome are reported to understand the contribution of each component to the overall treatment effect.

4.11. Subgroup analysis

For exploratory purposes, subgroup analyses for the primary endpoint will be conducted, to assess whether the treatment effect differs according subgroups. The following subgroups will be created:

- Effect according to study center
- Left ventricular ejection fraction<50% versus ≥50%
- Baseline estimated glomerular filtration rate (eGFR) <45 mL/min/1.73m² versus ≥45 mL/min/1.73m²
- Loop diuretic home maintenance dose ≤1 mg bumetanide, ≤10 mg torsemide, or ≤40 mg furosemide versus >1 mg bumetanide, >10 mg torsemide, or >40 mg furosemide

Net treatment benefits with 95% confidence intervals will be presented for each subgroup and tested against the null hypothesis of no effect in each subgroup with a significance level of 5%. A forest plot will be used to summarize the results visually.

4.12. Handling of missing data

The proposed statistical model assumes the missing data mechanism to be missing at random. If the level of missing data for the primary outcome in the modified intention-to-treat population exceeds 5%, 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.

4.13. Analysis of the secondary outcomes

To avoid inflation of the type I error, secondary outcomes will be tested in a hierarchical order. If the primary endpoint or any of the secondary endpoints is not statistically significant, effect sizes with 95% confidence intervals will be reported for the subsequent secondary outcomes, yet no formal statistical testing will be performed and no p-values will be reported. All statistical models implemented for the analysis of the secondary outcomes are mixed-effects models, including a fixed treatment effect and a random centre effect. For outcomes measured at multiple time-points, random patient effects (intercept and slope) are also included.

Secondary outcome 1: Relative NTproBNP decrease from baseline to 30 days after randomization [%]

For descriptive purposes, the geometric mean difference is reported with a 95% confidence interval with a fixed treatment effect, a random centre effect and random patient effects for intercept and slope.

Secondary outcome 2: Relative CA-125 decrease from baseline to 30 days after randomization [%]

For descriptive purposes, the geometric mean difference is reported with a 95% confidence interval with a fixed treatment effect, a random centre effect and random patient effects for intercept and slope.

Secondary outcome 3: Length of intravenous diuretic therapy [days]

For each treatment arm mean, median and interquartile range for the length of intravenous diuretic therapy will be presented for patients who survived to hospital discharge. The effect of treatment on length of intravenous diuretic therapy, for patients who survived to hospital discharge, is compared among treatment arms with a linear mixed model (fixed treatment effect and random centre effect). A log transformation will be employed as the model assumptions (i.e., normality) will likely be violated. Model assumptions will be investigated by means of diagnostic plots. The results of this model will be presented as (geometric) mean length of intravenous diuretic therapy (and 95% confidence interval) per treatment group, the rate ratio, 95% confidence interval and associated p-value.

Secondary outcome 4: Length of the index hospital admission [days]

For each treatment arm mean, median and interquartile range for the length of the index hospital admission will be presented for patients who survived to hospital discharge. The effect of treatment on length of the index hospital admission, for patients who survived to hospital discharge, is compared among treatment arms with a linear mixed model (fixed treatment effect and random centre effect). A log transformation will be employed as the model assumptions (normality) will likely be violated. Model assumptions will be investigated by means of diagnostic plots. The results of this model will be presented as (geometric) mean length of index hospitalization (and 95% confidence interval) per treatment group, the rate ratio, 95% confidence interval and associated p-value.

Secondary outcome 5: Successful decongestion defined as no more than trace oedema, absence of jugular venous distension and no rales upon the moment of transition from intravenous diuretics to oral maintenance therapy

For descriptive purposes, the number of patients in each treatment arm (percentages) who have successful decongestion are reported. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary

outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as an odds ratio with 95% confidence interval and the associated p value.

Secondary outcome 6: 5-point Likert scale for overall well-being assessed upon the transition from intravenous diuretics to oral maintenance therapy compared to the moment of randomization (much improved/slightly improved/neutral/slightly worse/much worse)

For descriptive purposes, the number of patients in each treatment arm (percentages) per category of overall well-being are reported. To investigate the treatment effect on the overall well-being, a generalized linear mixed model for an ordinal outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as odds ratios with 95% confidence interval and the associated p value.

Secondary outcome 7: Doubling of the serum creatinine or plasma cystatin C compared to baseline with an absolute value >2 mg/dL or >2 mg/L, respectively, or the need for ultrafiltration and/or renal replacement therapy during the index hospital admission (renal safety endpoint)

For descriptive purposes, the number of patients in each treatment arm (percentages) who meet the criteria for the renal safety endpoint are reported. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as an odds ratio with 95% confidence interval and the associated p value.

Secondary outcome 8: Systolic blood pressure <90 mmHg or mean arterial pressure <65 mmHg or need for vasopressors and/or inotropes during the index hospital admission (hemodynamic safety endpoint)

For descriptive purposes, the number of patients in each treatment arm (percentages) who meet the criteria for the hemodynamic safety endpoint are reported. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as an odds ratio with 95% confidence interval and the associated p value.

Secondary outcome 9: Death, non-elective rehospitalization or non-elective medical contact

For descriptive purposes, the number (%) of failure and success for the combined outcome of all-cause mortality, non-elective rehospitalizations, and non-elective medical contacts during the 30-day follow-up period will be given per treatment arm. To investigate the treatment effect on the occurrence of the combined endpoint, a generalized linear mixed model for a binary outcome will be employed. The model will incorporate a fixed treatment effect and random centre effect. The results of this model will be presented as an odds ratio, 95% confidence interval and the associated p value. If the treatment effect on this composite endpoint turns out to be statistically significant, the analysis will be performed for the composite endpoint of death and non-elective rehospitalization.