

**Randomized, controlled interventional trial to investigate
the efficacy of amiloride for the treatment of oedema in
human nephrotic syndrome (AMILOR)**

Statistical Report (SR)

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Note

This SR was performed according to the SOP BI07 (valid since 01.04.2022) of the ZKS Tübingen. The analysis tables and listings will be independently validated by a second statistician according the SOP BI06 (valid since 01.04.2022) of the ZKS Tübingen.

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Abbreviations

AE	Adverse Event
BCM	Body Composition Monitor, Fresenius Medical Care AG & Co
CRF	Case Report Form
ECW	Extracellular water
ENaC	Epithelial sodium channel
FCBP	Females of childbearing potential
FSH	Follicle stimulating hormone
GFR	Glomerular filtration rate
HbA1c	Haemoglobin A1c
HCT	Hydrochlorothiazide
IKEAB	Institut für Klinische Epidemiologie und angewandte Biometrie Tübingen
KDIGO	Kidney Disease – Improving Global Outcomes
NKCC2	Sodium-potassium-chloride cotransporter
NYHA	New York Heart Association
OH	Overhydration
RAAS	Renin-angiotensin-aldosterone system
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SR	Statistical Report
TMF	Trial Master File
WOCBP	Women of childbearing potential
ZKS	Zentrum für Klinische Studien Tübingen

1 Introduction

This SR is based on the study protocol Version 1.1 as of 10. January 2020 with amendment 1 as of 01. March 2021 (EudraCT-No: 2019-002607-18), and the Statistical Analysis Plan (final version as of 17. November 2023). Text citation directly from the study protocol is given in *Italic letters*.

Note: Due to small recruitment the study was terminated after n = 20 patients.

1.1 Preface

The nephrotic syndrome is characterized by a high ("nephrotic") proteinuria > 3.5 g / day with resulting hypoalbuminemia, as well as by oedema and hyperlipidaemia. Affected patients suffer from massive generalized oedema involving the face and eyelids, effusions into the body cavities pleura, peritoneum and rarely pericardium as well as increased body weight.

In addition to the disease specific treatment commonly involving immunosuppression, supportive therapy of patients with acute nephrotic syndrome includes reduction of proteinuria by the renin-angiotensin-aldosterone-system (RAAS) blockade, blood pressure control, lipid lowering, thromboembolic prophylaxis and the correction of volume overload, which is achieved by a dietary salt restriction and diuretic therapy.

Currently, loop diuretics are preferred as they have the highest diuretic potency. However, the diuretic effect of loop diuretics is lower in nephrotic syndrome than in other forms of oedema or in healthy volunteers. A possible cause of this resistance to loop diuretics could be the activation of the epithelial sodium channel (ENaC) that is located distally to the sodium-potassium-chloride cotransporter (NKCC2), the target of loop diuretics. Sodium retention via ENaC may therefore counteract the inhibition of NKCC2.

To overcome refractory oedema, either higher doses of loop diuretics or combination with thiazide diuretics (e.g., hydrochlorothiazide, HCT) for sequential nephron blockade or with distally acting diuretics such as the ENaC inhibitors amiloride and triamterene blockers are conceivable.

Additionally, our investigations in patients with protein uric kidney disease and nephrotic syndrome show that activation of ENaC by serine proteases also appears to be of central importance as a mechanism of oedema formation in humans

1.2 Purpose of the Study

Treatment of resistant oedema and overhydration in nephrotic syndrome can be challenging and we successfully use ENaC inhibitors (in combination with hydrochlorothiazide) in these patients. However, most clinicians treat nephrotic oedema with higher doses of loop diuretics, or combination therapy with thiazide diuretics. Worldwide, ENaC inhibitors are rarely used in nephrotic syndrome. This is due to lack of evidence of the role of activated ENaC and the effectiveness of ENaC inhibitors in human nephrotic syndrome. In this clinical trial, we aim to investigate the therapeutic effect of amiloride on the reduction of oedema and overhydration in the nephrotic syndrome.

2 Study Objectives

In the study, patients with nephrotic syndrome will be randomized to medication with the investigational drug amiloride (study arm 1) or the comparator furosemide (study arm 2) for a study period of 16 days. In case of treatment resistant oedema, hydrochlorothiazide (HCT) is added at day 8 in both study arms. To prevent bias, patients included in the study are randomized to start treatment with amiloride or furosemide.

2.1 Primary Objectives and Endpoint

In the light of our current results on ENaC activation by serine proteases aberrantly filtered into urine in nephrotic syndrome, we hypothesize that targeting ENaC with amiloride will lead to a more effective reduction of overhydration in nephrotic syndrome than standard treatment with the loop diuretic furosemide.

Primary objective of the study is therefore to prove the efficacy and superiority of amiloride for reduction of oedema and overhydration (OH) in human nephrotic syndrome in comparison to standard medication with furosemide. Primary endpoint of the study is the decrease of overhydration after 8 days, compared to baseline. OH is measured using bioimpedance spectroscopy with the device Body Composition Monitor, Fresenius Medical Care AG & Co; BCM) and expressed as percent of extracellular water (% ECW).

2.2 Secondary Objectives and Endpoints

Secondary endpoints represent further parameters to evaluate course of overhydration and regulation of body volume status after initiation of study medication with amiloride or furosemide. Secondary endpoints include

- 1. decrease of OH after 16 days, measured using bioimpedance spectroscopy and expressed as % ECW*
- 2. decrease of body weight after 8 and 16 days*
- 3. decrease of oedema circumference after 8 and 16 days, measured as the largest extent on the lower leg*
- 4. decrease of systolic and diastolic blood pressure after 8 and 16 days*
- 5. increase of urine volume and natriuresis after 8 and 16 days, determined from urine collection over 24 hours*
- 6. course of plasma renin activity and serum aldosterone concentration after 8 and 16 days*
- 7. number of required changes of dose of study medication*
- 8. need for co-medication with HCT after 8 days*
- 9. occurrence of adverse events*

3 Study Methods

3.1 General Study Design and Plan

The study design is a phase IIIb, monocentre, interventional, two-arm, randomized, open-label controlled clinical trial. We decided for a study type with randomization as method against bias and have omitted blinding, as laboratory results required for safety reasons and dose adjustments will inevitably reveal to which study group patients have been randomized.

Overall study design is shown in figure 1.

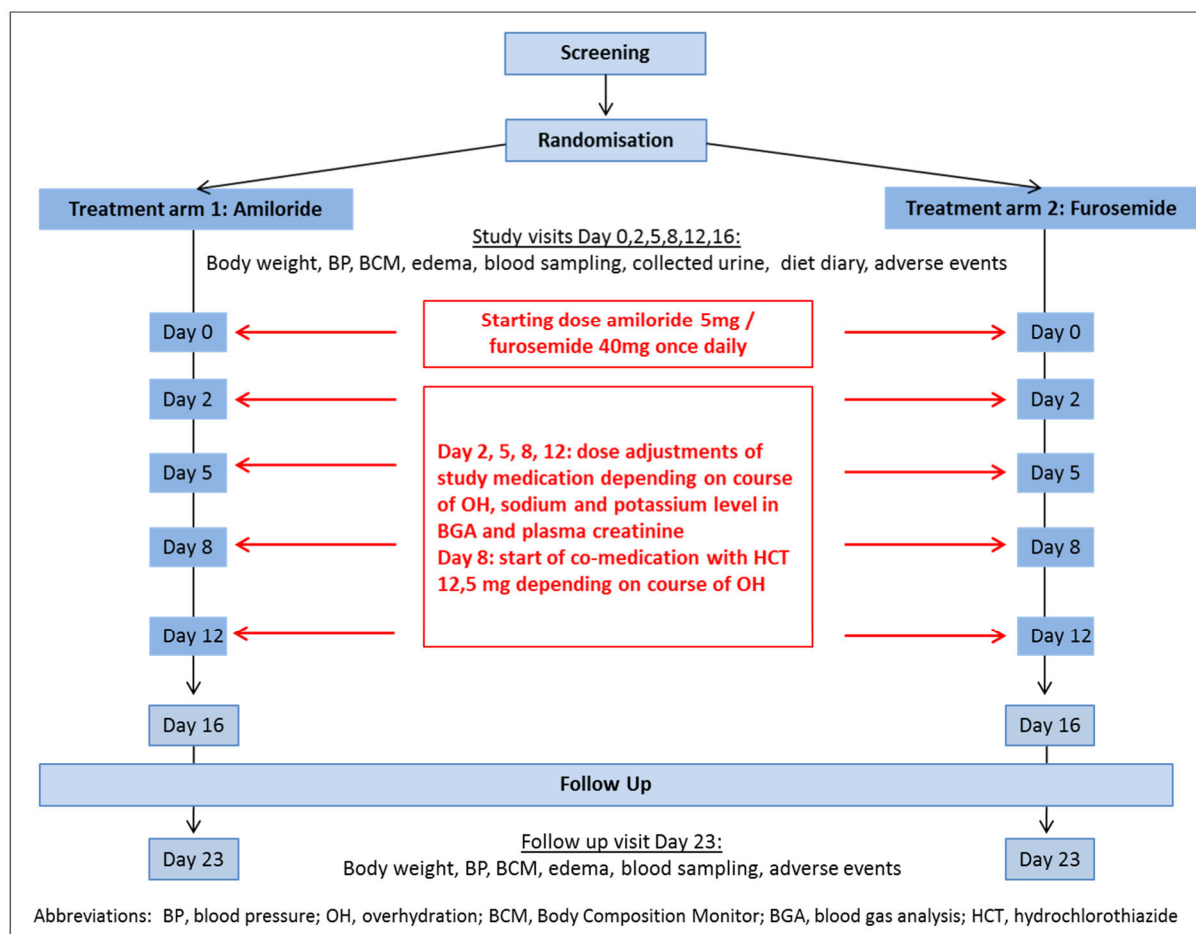


Figure 1 Overall study design (according to study protocol)

3.2 Study Treatment

Participants are randomized to start medication with amiloride 5 mg (trial arm 1) or furosemide 40 mg (trial arm 2) at day 0. Dose adjustments depending on course of overhydration, sodium and potassium level in blood gas analysis and plasma creatinine are performed on day 2, 5, 8 and 12. In case of therapy-refractory overhydration, co-medication with HCT 12,5 mg is started at day 8.

3.3 General Study Population and Inclusion/Exclusion Criteria

This study will include patients with acute nephrotic syndrome (definition see below) due to different underlying diseases (e.g. diabetic nephropathy, membranous nephropathy, minimal change disease, focal segmental glomerulosclerosis). The patients will be recruited from the nephrological outpatient department of the University Hospital Tuebingen or after referral from local nephrological practices.

Adult male and female patients with acute nephrotic syndrome and fulfilling the below outlined inclusion criteria will be enrolled into the study.

Trial population will consist of both genders. Gender distribution in the trial is supposed to reflect the distribution in the real patient's population, i.e. there will be no prior defined quantitative ratio between females and males. No statistical analysis based on genders or differences between genders will be performed.

3.3.1 Inclusion Criteria

According to the study protocol subjects meeting all of the following criteria will be considered for admission to the trial:

- 1. Acute nephrotic syndrome with proteinuria > 3 g/day and formation of oedema.*
- 2. Age ≥ 18 years at the time of signing the informed consent.*
- 3. Understand and voluntarily sign an informed consent document prior to any study related assessments/procedures.*
- 4. Ability to adhere to the study visit schedule and other protocol requirements.*
- 5. Use of adequate thrombosis prophylaxis due to the increased risk of thrombosis in nephrotic syndrome and the expected fluctuations in volume balance during study participation*
- 6. Subject (male or female) is willing to use highly effective methods of contraception according to the "Clinical trial fertility group" recommendations (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf) during treatment and for 28 days (male or female) after the end of treatment (adequate: intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner¹, sexual abstinence²; due to the increased risk of thrombosis in nephrotic syndrome, no hormonal contraceptives are recommended).*
- 7. Female Patients of childbearing potential (WOCBP)³ must agree to pregnancy testing before inclusion in the study.*
- 8. Female Patients must agree to abstain from breastfeeding during study participation and 28 days after study drug discontinuation.*
- 9. All subjects must agree not to share medication.*

¹ Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success

² In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject

³ For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

3.3.2 Exclusion Criteria

Subjects presenting with any of the following criteria will not be included in the trial:

1. Severe reduction of kidney function: Creatinine clearance or calculated GFR < 30 mL/min/1.73m² or acute kidney injury KDIGO stage 2 or 3 or anuria.
2. Hypovolemia or dehydration.
3. Uncontrolled diabetes mellitus.
4. Hypotension, systolic blood pressure < 90 mm Hg.
5. Hyperkalaemia, plasma potassium concentration > 4.8 mmol/l.
6. Hypokalaemia, plasma potassium concentration < 3.3 mmol/l.
7. Hyponatremia, plasma sodium concentration < 128 mmol/l.
8. Hypercalcemia, ionized calcium > 2.0 mmol/l or total albumin corrected calcium > 3.0 mmol/l.
9. Signs of cardiac decompensation (orthopnoea, dyspnoea NYHA IV).
10. Hepatic coma or precoma.
11. Symptoms of gout.
12. Current therapy with potassium-sparing diuretics (e.g. spironolactone) or potassium supplements.
13. Women during pregnancy and lactation.
14. History of hypersensitivity to the investigational medicinal product, comparator or co-medication or to any drug with similar chemical structure or to any excipient present in the pharmaceutical form of the investigational medicinal product, comparator or co-medication.
15. Any other clinical condition that would jeopardize the patient's safety while participating in this clinical trial.
16. Active participation in other clinical trials or observation period of competing trials.

3.4 Randomisation

The randomization will follow a randomization scheme of a 1:1 (amiloride and furosemide). Subjects will be assigned to their respective treatment by randomization. A randomisation number will be assigned (= Random. number). Subjects will then receive their study medication during their personal visit at day 0 of study participation.

3.5 Blinding

The principal investigator and the study team have opted against blinding, as laboratory results required for safety reasons and for definition of dose adjustments will inevitably reveal to which study group each patient was randomized (hypokalaemia – furosemide, hyperkalaemia – amiloride).

3.6 Study Procedures

This Study consists of the following consecutive phases:

- Study entry
- Treatment
- Follow-up.

Time-points and trial procedures are listed in Table 1.

Table 1 Table of Events

	Study entry	Treatment						Follow-up
Visit number	1	2	3	4	5	6	7	8
Day	-3 to -1	0	2	5	8	12	16	23
Study Entry								
Informed Consent	X							
Demographics, including sex, body weight, high, age	X							
Inclusion/Exclusion criteria: anamnesis, clinical examination, blood pressure, pre-existing medication, proteinuria in 24 hours collected urine, general laboratory control (including blood count, creatinine, urea, liver enzymes, lactate dehydrogenase, c-reactive protein, electrolytes (potassium, sodium, calcium), glucose, HbA1c, pregnancy test in females)	X							
Safety Assessments								
Adverse events		X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X
Check of the study medication		X	X	X	X	X	X	
Return of any remaining study medication							X	
Plasma creatinine	X	X	X	X	X	X	X	X
Potassium and sodium level in blood gas analysis	X	X	X	X	X	X	X	X

Continued Table 1 Table of Events

	Study entry	Treatment						Follow-up
Visit number	1	2	3	4	5	6	7	8
Day	-3 to -1	0	2	5	8	12	16	23
Efficacy Assessment								
Body weight	X	X	X	X	X	X	X	X
Bioimpedance spectroscopy with BCM	X	X	X	X	X	X	X	X
Oedema circumference	X	X	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X	X	X
Plasma renin activity, serum aldosterone concentration	X	X	X	X	X	X	X	X
24 hours urine volume and natriuresis	X		X	X	X	X	X	
Diet diary during urine collection	X		X	X	X	X	X	
Check for dose adjustment		X	X	X	X	X		

4 Sample Size

Primary endpoint of the study is decrease of overhydration (measured using bioimpedance spectroscopy) after 8 days, compared to baseline. Based on data from a baseline sample ($n = 14$) and clinical experience on reduction of overhydration baseline overhydration is expected to be 26.5 % of extracellular water (standard deviation 12.3; normal distribution is assumed) and it is expected that the overhydration will be reduced by 90 % in the amiloride group and by 50 % in the furosemide group. For the final t-test for two groups, a one-tailed significance level of 0.05 and a power of 80 % should be ensured. From these conditions results a sample size of $n = 18$ patients per group (total $n = 36$; calculated for a t-test with the nQuery® Advisor 7.0 program). In order to take dropouts into account, the sample size is defined as $n = 22$ per group (total $n = 44$).

5 General Considerations

5.1 Timing of Analyses

The analysis was performed after finalization and approval of the SAP (final version xx.11.2023). After the close-out visits took place the final analysis was performed on the data which have been declared clean according to ZKS-SOP DA05.

5.2 Analysis Population

5.2.1 Intention-to-treat Population (ITT)

According to study protocol and SAP all statistical analyses based on the Intention-to-Treat Population (ITT). The ITT includes all randomized patients with exception of patients who withdraw their informed consent for the analysis of their data during the study.

5.2.2 Safety Population (Safety)

The safety population includes all subjects who received any study treatment.

6 Data and Data Management

6.1 Case Report Form

The trial case report form (CRF) was the primary data collection instrument for the trial. All data requested on the CRF must be recorded. All missing data must be explained.

For this project, paper Case Report Forms (CRFs) were used. The investigator is responsible for ensuring that all sections of the CRF are completed correctly and that entries can be verified against source data. The correctness of entries in CRFs was confirmed by dated signature of the responsible investigator.

6.2 Data Handling

After first check for plausibility by eye, all data were entered in a database as recorded in the CRF. To ensure data quality a double data entry was performed. After completion of data entry, checks for plausibility, consistency, and completeness of the data were performed. Based on these checks, queries were produced combined with the queries generated by visual control.

6.3 Missing Data

All variables included in the CRF are mandatory. The monitoring assures quality of the assessments. Thus, missing values were expected only due to refusal by patients. In the analysis of the primary endpoint no missing values occurred. All other missing data were given in the descriptive tables but excluded from the statistical test.

6.4 Derived Variables

Endpoints that are derived variables, i.e. not collected via the CRF:

- Change of overhydration from baseline to day 8 (see primary endpoint)
- Change of overhydration from baseline to day 16
- Change of body weight, oedema circumference, systolic and diastolic blood pressure after 8 and 16 days
- Change of urine volume, natriuresis, plasma renin activity and serum aldosterone concentration after 8 and 16 days

7 Statistical Analyses

7.1 Study Information

For all study subjects the disposition during the study and the duration including reasons (death, toxicity, treatment failure, withdraw consent) for pre-termination was shown. All data for the CONSORT 2010 flow diagram were given, i.e. the allocation to the analysis population and the number of subjects dropped out.

7.2 Protocol Deviations

The specific protocol deviations during the randomized treatment phase were reviewed in the blind data review regarding the impact for the confirmatory analysis and categorized into major and minor. For all major protocol deviations summary statistics were produced.

7.2.1 Major Protocol Deviations

The following events were classified as major protocol deviations:

- *Deviation against inclusion or exclusion criteria*
- *Missing visit 5 (day 8) → no calculation of primary endpoint possible*
- *Late visit until day 8*

7.2.2 Minor Protocol Deviations

The following events were classified as minor protocol deviations:

- *Replacement of missing laboratory values from clinical patient record*
- *Late follow-up visit (1 – 5 days)*
- *Due to practical reasons blood and urine samples were taken at the routine ambulance visit which was declared as inclusion visit, and took place before the formal informed consent → no consequences for patient safety or data integrity.*
- *Stop of increasing the dose.*

According to the blind data review (finalized 02.11.2023) no major protocol deviations occurred. Note-to-files were documented during the course of the study for all minor protocol deviations.

7.3 Descriptive Statistics

For all study data descriptive summary statistics were produced. Descriptive analysis include absolute and percentage frequencies for categorical variables, means, medians, standard deviations, quartiles and ranges for quantitative variables and medians, quartiles and ranges for ordinal variables.

7.4 Efficacy Analyses

7.4.1 Primary Endpoint

The primary endpoint variable is the change of overhydration (OH) after 8 days compared to baseline, measured by bioimpedance spectroscopy using the Body Composition Monitor and expressed in percent of extracellular water (% ECW). The primary endpoint variable was compared between the two study arms, which are patients with amiloride treatment and patients with furosemide treatment.

The null-hypothesis is that there is equal or greater decrease of OH after 8 days in the group of patients with furosemide treatment compared to the group of patients with amiloride treatment. The alternative hypothesis is that there is a greater decrease of OH after 8 days in the group of patients with amiloride treatment compared to the group of patients with furosemide treatment. For analysis of the primary endpoint variable a one-sided t-test for two groups was performed to test the null hypothesis against the alternative hypothesis. Effect size and 95%-confidence interval for effect size were estimated. Only the rejection of the null hypothesis was interpreted as statistical evidence for the efficacy of the amiloride treatment.

7.4.2 Secondary Endpoints

Secondary endpoint variables are listed in section 2.2. All secondary endpoints were compared and statistically assessed for descriptive purposes and not in a confirmatory sense. The aim of the analysis was explorative data analysis, not hypothesis testing or generation of evidence for efficacy and no attempt was made to adjust the p-values of statistical tests of the secondary endpoints for multiple testing. If adequate, secondary endpoints were compared and statistically assessed using t-tests for two groups or covariance techniques with baseline values as covariates in case of quantitative variables. Depending on distribution, non-parametric tests were indicated. Dichotomous data were compared and statistically assessed using Mantel-Haenszel chi-squared tests including relative risks and 95%-confidence intervals for relative risks.

7.5 Safety Analysis

Safety was assessed by frequency tabulations and line listings for AEs and SAEs.

8 Technical Details

At the time of writing this SR the study specific documents used data base were stored at Koordobas 5.1.0, the software package for statistical analysis was SAS 9.4, and the operating system of the computer was Windows Server 2019.

9 Results

9.1 Study Information

For this study $n = 20$ patients (table 1.1 in appendix 1) were assessed for eligibility before study termination due to small recruitment. All patients fulfil the inclusion criteria and did not violate against one of the exclusion criteria (see tables 1.2 in appendix 1). None of the patients withdraw his/her informed consent for participating furthermore and for analysis of his/her data during the study.

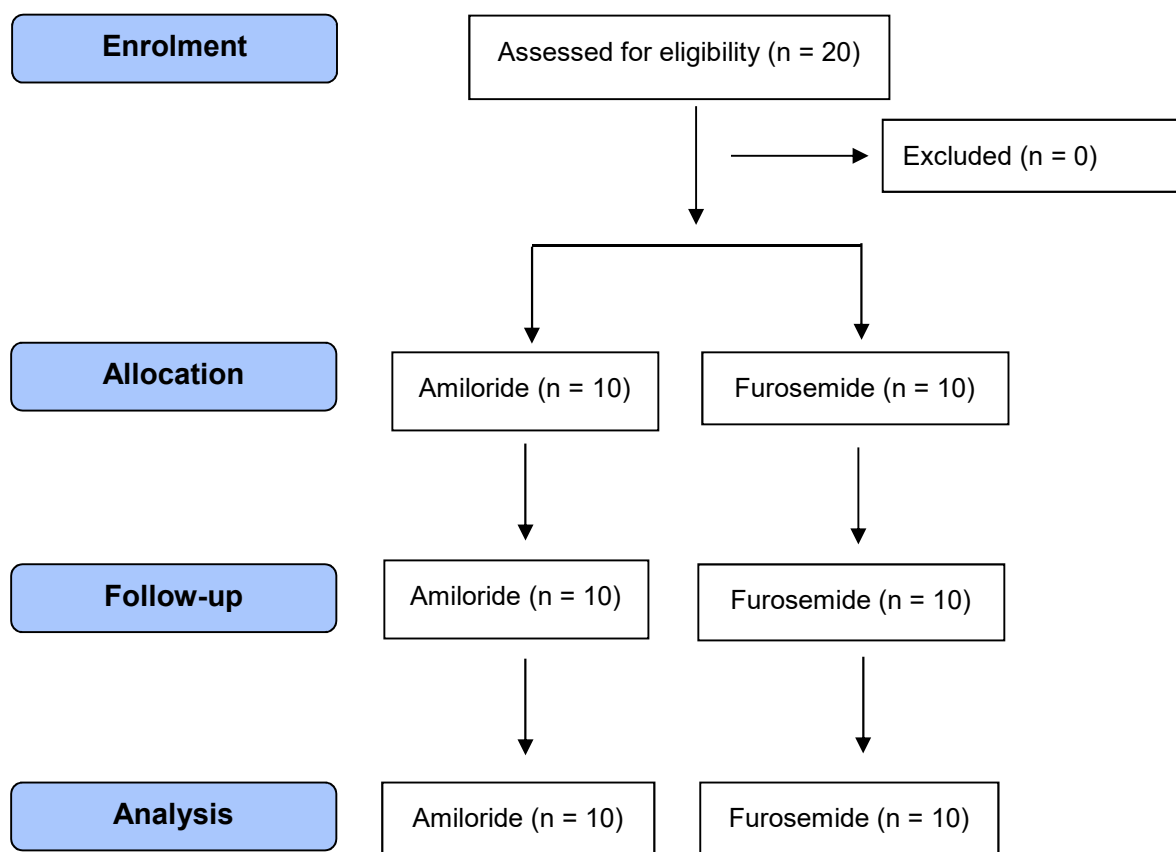


Figure 2 CONSORT 2010 Flow chart for analysis population

All patients participated in 5 (until day 8) of 8 visits (see table 1.3 in appendix 1), during the two last treatment visits and follow-up visit (visit 6 – visit 8) for one patient treatment was not continued (physician's decision). Overall, no drop-out occurred.

Mean study duration (visit 1 = study entry until visit 5 = day 8) for the patients was 8.0 ± 0 days, mean time from study entry to end of study was 24.5 ± 5.0 days (see table 1.4 in appendix 1). Due to insufficient recruitment rate the trial was terminated (see section 7.7 study protocol amendment 1 as of 01. March 2021), reasons for termination for the patients are given in table 1.5 in appendix 1.

No major protocol deviation, as defined in section 7.2.1, occurred during the study. For one patient (Pat-ID 2) the time between study entry and first treatment was 4 days (-3 to -1 day were planned, see Table 1).

9.2 Descriptive Analysis

The descriptive analysis was performed according to section 7.3. All continuous variables and categorical measures are shown in summary tables, which were structured with a column for each part in the order (Amiloride, Furosemide, all) and were annotated with the respective population size, including any missing observations/values. The results of the descriptive analysis are shown in appendix 1 (see table 2).

Table 2 Results of descriptive analysis – Overview of tables within appendix 1

Study course (visit)	Parameter	Number
Study entry (visit 1)	Demographic characteristics	2.1.1
	Clinical examination	2.1.2
	Blood analysis	2.1.3
	Plasma renin and Aldosterone	2.1.4
	Urine analysis	2.1.5
	Medical history	2.1.6
	Previous medication	2.1.7
Treatment phase (visit 2 – 8)	Clinical examination (incl. BCM)	2.2.1
	Day 0 – day 23	2.2.1.1 – 2.2.1.5
	Blood analysis (incl. blood gas analysis)	2.2.2
	Creatinine, sodium, potassium	2.2.2.1 – 2.2.2.3
	Plasma renin and Aldosterone	2.2.3
	Day 0 – day 23	2.2.3.1 – 2.2.3.2
	Urine analysis	2.2.4
	Volume, total protein, sodium, potassium	2.2.4.1 – 2.2.4.4
	Study medication: review of dose adjustment	2.2.5
	Day 0, day 2, day 5, day 8, and day 12	2.2.5.1 – 2.2.5.5
	Concomitant medication	2.2.6

9.3 Statistical Analysis

9.3.1 Primary Endpoint

According to the study protocol *the primary objective is to prove the efficacy and superiority of amiloride for reduction of oedema and overhydration (OH)*, measured by percent of extracellular water (% ECW). Therefore, the primary endpoint is the change of OH after 8 days, compared to baseline. *The primary endpoint variable was compared with a one-sided t-test for two groups between the two study arms, which are patients with amiloride treatment and patients with furosemide treatment. Additionally, effect size and 95%-confidence interval for effect size were estimated.*

Normal distribution was founded for % ECW at baseline, after 8 day, and for the difference between baseline and day 8 (Shapiro-Wilk test $p = 0.8181$, $p = 0.2553$, $p = 0.4196$). Therefore, the planned t-test was sustainable for comparison of the two groups amiloride and furosemide. Results of the distributions of % ECW at baseline, day 8 and difference baseline – day 8 are shown in table 3.

Table 3 Change of overhydration form baseline to day 8

% ETCW	N		Mean	Sd.	Min.	Max.	Percentile		
	Valid	Miss					25	Median	75
Baseline									
All	20	0	26.21	7.81	10.10	39.70	21.85	27.85	30.90
Amiloride	10	0	25.80	10.08	10.10	39.70	15.50	26.35	35.10
Furosemide	10	0	26.61	5.19	17.50	35.40	24.10	27.85	29.40
Day 8									
All	20	0	21.77	8.68	9.60	38.10	14.30	19.90	28.10
Amiloride	10	0	20.93	7.66	9.60	32.60	15.50	19.20	26.80
Furosemide	10	0	22.60	9.94	12.00	38.10	12.40	21.15	28.50
Difference Baseline – day 8									
All	20	0	4.44	6.05	-10.30	15.80	0.850	3.80	7.35
Amiloride	10	0	4.87	5.74	0	15.80	0.800	1.95	6.40
Furosemide	10	0	4.01	6.62	-10.30	11.90	0.900	5.15	8.30

Overall, no difference for the change after 8 days was detected between the study groups amiloride and furosemide ($p = 0.380$ one-sided t-test), calculated effect size = 0.1388, 95%-CI -0.7446 – 1.0104. Therefore, between amiloride and furosemide no difference was shown for the change of overhydration within 8 days.

9.3.2 Secondary Endpoints

As given in section 7.4.2 exploratory analysis of the secondary endpoints, given in section 2.2, was performed. Results are shown in tables 3.1 – 3.2.

Table 3.1 Secondary endpoints – quantitative parameters

Parameter	N		Mean	Sd.	Min.	Max.	Percentile		
	Valid	Miss					25	Median	75
Change of overhydration (%ECW) within 16 days (p = 0.358)*									
All	19	1	8.932	9.947	-4.200	35.900	1.300	7.500	13.100
Amiloride	9	1	11.211	12.933	-4.200	35.900	1.300	10.100	14.400
Furosemide	10	0	6.880	6.272	-3.400	16.800	2.800	7.400	10.100
Change of body weight [kg] within 8 days (p = 0.546)*									
All	20	0	1.765	2.539	-2.700	5.800	0.350	1.850	4.000
Amiloride	10	0	2.120	2.537	-1.400	5.800	0.700	1.550	4.600
Furosemide	10	0	1.410	2.624	-2.700	4.800	0.000	1.850	3.700
Change of body weight [kg] within 16 days (p = 0.667)*									
All	19	1	3.368	4.598	-7.600	12.80	1.500	2.600	6.700
Amiloride	9	1	3.867	5.576	-7.600	12.800	1.700	3.800	7.200
Furosemide	10	0	2.920	3.765	-3.400	10.300	1.500	2.000	4.600

* t test, two-sided

Continued Table 3.1 Secondary endpoints – quantitative parameters

Parameter	N		Mean	Sd.	Min.	Max.	Percentile		
	Valid	Miss					25	Median	75
Change of edema circumference [cm] right within 8 days (p = 0.500)**									
All	20	0	0.925	3.256	-6.000	11.500	-0.550	0.250	2.000
Amiloride	10	0	1.190	4.490	-6.000	11.500	-0.600	0.000	3.000
Furosemide	10	0	0.660	1.438	-1.400	3.000	-0.500	0.500	2.000
Change of edema circumference [cm] right within 16 days (p = 0.065)**									
All	19	1	1.021	3.466	-8.500	6.000	0.500	1.500	3.000
Amiloride	9	1	1.767	4.253	-8.500	6.000	2.000	2.500	3.000
Furosemide	10	0	0.350	2.625	-5.000	3.500	0.000	0.750	1.500
Change of edema circumference [cm] left within 8 days (p = 0.410)**									
All	20	0	1.070	2.974	-5.500	10.800	-0.250	0.550	2.000
Amiloride	10	0	1.290	4.057	-5.500	10.800	0.000	0.550	2.000
Furosemide	10	0	0.850	1.454	-1.000	3.000	-0.500	1.000	2.000
Change of edema circumference [cm] left within 16 days (p = 0.102)**									
All	19	1	1.137	3.871	-9.500	7.000	0.000	2.000	3.500
Amiloride	9	1	1.844	4.706	-9.500	7.000	1.000	3.000	4.000
Furosemide	10	0	0.500	3.055	-5.000	4.500	-1.000	1.500	2.500
Change of systolic blood pressure [mm Hg] within 8 days (p = 0.766)**									
All	20	0	11.90	26.66	-40.00	97.00	-1.50	7.50	20.50
Amiloride	10	0	13.30	33.90	-40.00	97.00	1.00	10.00	14.00
Furosemide	10	0	10.50	18.62	-8.00	48.00	-5.00	4.00	24.00
Change of systolic blood pressure [mm Hg] within 16 days (p = 0.840)**									
<i>Difference Baseline – day 8</i>									
All	19	1	14.68	25.25	-47.00	84.00	2.00	15.00	22.00
Amiloride	9	1	14.22	34.40	-47.00	84.00	-2.00	15.00	22.00
Furosemide	10	0	15.10	14.94	-1.00	51.00	6.00	12.50	15.00
Change of diastolic blood pressure [mm Hg] within 8 days (p = 0.599)*									
All	20	0	3.40	13.10	-19.00	36.00	-4.50	4.50	12.50
Amiloride	10	0	5.00	15.13	-19.00	36.00	3.00	4.50	12.00
Furosemide	10	0	1.80	11.31	-18.00	16.00	-6.00	4.50	13.00
Change of diastolic blood pressure [mm Hg] within 16 days (p = 0.511)*									
All	19	1	7.00	12.12	-17.00	30.00	-1.00	5.00	18.00
Amiloride	9	1	9.00	14.53	-17.00	30.00	0.00	9.00	18.00
Furosemide	10	0	5.20	9.94	-6.00	22.00	-2.00	2.500	13.00
Change of urine volume [ml/24 h] within 8 days (p = 0.221)**									
All	17	3	564	1668	-1200	6580	-100	350	694
Amiloride	8	2	1053	2271	-500	6580	100	375	698
Furosemide	9	1	128	775	-1200	1250	-300	150	694
Change of urine volume [m/24 hl] within 16 days (p = 0.994)*									
All	16	4	459	622	-300	1680	11	300	690
Amiloride	7	3	457	626	-300	1680	21	300	600
Furosemide	9	1	460	657	-200	1600	0	200	780

* t test, two-sided, ** Wilcoxon test two-sided

Continued Table 3.1 Secondary endpoints – quantitative parameters

Parameter	N		Mean	Sd.	Min.	Max.	Percentile		
	Valid	Miss					25	Median	75
Change of natriuresis within 8 days (<i>p</i> = 0.559)*									
All	19	1	-2.37	4.78	-10.00	8.00	-6.00	-2.00	1.00
Amiloride	10	0	-3.00	3.71	-9.00	1.00	-6.00	-3.00	1.00
Furosemide	9	1	-1.67	5.90	-10.00	8.00	-5.00	0.00	1.00
Change of natriuresis within 16 days [mm] (<i>p</i> = 0.818)*									
All	18	2	-2.67	3.91	-11.00	3.00	-6.00	-1.50	1.00
Amiloride	9	1	-2.44	3.09	-7.00	2.00	-5.00	-2.00	-1.00
Furosemide	9	1	-2.89	4.78	-11.00	3.00	-6.00	-1.00	1.00
Change of plasma renin activity within 8 days (<i>p</i> = 0.260)**									
All	20	0	-5.19	13.28	-59.20	0.80	-3.50	-0.90	-0.10
Amiloride	10	0	-9.01	18.35	-59.20	0.80	-9.30	-1.75	-0.20
Furosemide	10	0	-1.37	1.81	-5.20	0.20	-2.30	-0.55	0.00
Change of plasma renin activity within 16 days (<i>p</i> = 0.153)**									
All	18	2	-7.08	14.80	-59.90	0.80	-6.90	-1.50	-0.40
Amiloride	8	2	-13.21	21.00	-59.90	0.80	-17.55	-4.85	-0.90
Furosemide	10	0	-2.18	3.25	-9.30	0.50	-1.60	-0.85	-0.40
Change of serum aldosterone concentration within 8 days (<i>p</i> = 0.041)**									
All	20	0	-48.50	94.29	-348.0	51.00	-104.0	-9.00	3.00
Amiloride	10	0	-82.50	115.82	-348.0	39.00	-146.0	-36.50	-5.00
Furosemide	10	0	-14.50	52.77	-113.0	51.00	-10.0	-3.00	19.00
Change of serum aldosterone concentration within 16 days (<i>p</i> = 0.008)**									
All	19	1	-87.53	121.62	-324.0	49.00	-186.0	-16.00	-3.00
Amiloride	9	1	-167.00	134.44	-324.0	49.00	-270.0	-186.00	-53.00
Furosemide	10	0	-16.00	38.94	-115.0	18.00	-16.0	-8.50	10.00

* t test, two-sided, ** Wilcoxon test two-sided

Table 3.2 Secondary endpoints – qualitative parameters

Parameter	Code	All (n = 20)		Amloride (n = 10)		Furosemide (n = 10)	
		%	n	%	n	%	n
Frequency of change of dose							
	0	5.0	1	10.0	1	0.0	0
	1	60.0	12	50.0	5	70.0	7
	2	10.0	2	10.0	1	10.0	1
	3	20.0	4	20.0	2	20.0	2
	5	5.0	1	10.0	1	0.0	0
Need of comedication with HCT after day 8							
	Day 8 – day 11	45.0	9	40.0	4	50.0	5
	Day 12 – day 15	60.0	12	60.0	6	60.0	6
	Day 8 – day 15	45.0	9	40.0	4	50.0	5
Number of AEs							
	0	30.0	6	50.0	5	10.0	1
	1	35.0	7	30.0	3	40.0	4
	2	15.0	3	10.0	1	20.0	2
	3	20.0	4	10.0	1	30.0	3

9.4 Safety Analysis

9.4.1 AEs and SAEs

The analysis population for the safety analysis included all subjects who received any study treatment (see SAP, section 5.2.2). Frequencies of AEs and SAEs are shown in table 4.1 and 4.2, and for individual patient within the listing (appendix 2).

Table 4.1 Adverse events (AEs)

Kind of AE	Duration [days]	Grade	Therapy	Change of IMP dose
<i>Amiloride</i>				
planned hospitalization for kidney biopsy	3	Mild	-	No
Harnwegsinfektion	11	Moderate	treatment with Ciprofloxacin 250 mg 1-0-1	No
Nausea, vomiting, diarrhea due to hypocalcemia due to hypoparathyroidism	4	Mild	Calcium + Vit D Supplementation	No
planned hospitalization for kidney biops	1	Mild	-	Not known
Ascites and pleural effusion	6	Moderate	Adjustment of study medication: increase of amiloride to 15 mg	Dose escalation
SARS-CoV2-Infektion	9	Mild	-	Not known
Nasenbluten	0	Mild	-	No
Übelkeit	1	Mild	-	No
SARS-CoV2-Infektion	4	Mild	Pause of tacrolimus medication	Not known
<i>Furosemide</i>				
Geplante stationäre Aufnahme zur Nierenpunktion	1	Mild	-	No
Leberwerte (Transaminasen) erhöht	9	Mild	-	No
planned hospitalization for kidney biopsy	1	Mild	-	No
elevated cholesterine and triglycerides	43	Mild	Atorvastatin	No
Krämpfe an Händen und Waden	11	Mild	-	Dose reduction
Geplante Nierenbiopsie	0	Mild	-	No
Schwindel	1	Moderate	-	No
SARS-CoV2-Infektion	8	Moderate	-	No
Kopfschmerzen	11	Mild	-	No
Nasenbluten	0	Mild	-	No
planned hospitalization for kidney biopsy	1	Mild	-	No
Ausschlag Rücken + Bauch	4	Mild	-	No

During the study course all patients with AEs were recovered and the AE was subsided.

Table 4.2 Serious adverse events (SAEs)

Kind of AE	Duration [days]	Grade	Therapy	Change of IMP dose
<i>Amiloride</i>				
delayed discharge from hospital, makrohämaturia after kidney biopsy	2	Mild	Blasenspülkatheter	No
<i>Furosemide</i>				
STEMI, Koronarangiographie	4	Life threatening	Koronarangiographie, Thrombozytenhemmung, Überwachung auf CPU bis 27.09.2021	No
Abdominal pain and diarrhea	8	Moderate	Analgetic therapy intensified, in-hospital treatment	No
Abdominal pain	7	Moderate	Analgetic therapy intensified, in-hospital treatment	No
Pericardial effusion, in-hospital monitoring	3	Moderate	-	No
Acute kidney injury	11	Severe	in-hospital treatment	No

During the study course all patients with SAEs were recovered and the SAE was subsided.

9.4.2 Safety criteria for dose adjustment

During the course of the study criteria for a break of study medication as well for reduction of study medication were recorded via plasma creatinine level, and plasma sodium level. Frequencies and reasons for break of or reduction of study medication are shown in table 4.3, and for individual patient within the listing (appendix 2).

Table 4.3 Safety criteria for dose adjustment

Dose adjustment	Visit	n	Kind of criteria
Pause: break of study medication until next study visit	Day 8	1	Kreatinin >5.3 oder <3.0 mmol/l (pCRF, V01)
	Day 12	2	Anstieg Kreatinin um >0.4 mg/dl seit Tag 0
Dose reduction: no study medication for the next day	Day 2	1	>4.8 oder <3.3 mmol/l (pCRF, V01)
		1	Abnahme OH seit Tag 0 mehr als 8.5 %ECW
		1	Systolischer RR < 90 mmHg oder diastolischer RR < 60 mm
	Day 5	2	>4.8 oder <3.3 mmol/l (pCRF, V01)
		1	Systolischer RR < 90 mmHg oder diastolischer RR < 60 mm
	Day 8	1	>4.8 oder <3.3 mmol/l (pCRF, V01)
		1	Abnahme OH seit Tag 0 mehr als 8.5 %ECW
	Day 12	1	>4.8 oder <3.3 mmol/l (pCRF, V01)
		1	Abnahme OH seit Tag 0 mehr als 8.5 %ECW
		1	Systolischer RR < 90 mmHg oder diastolischer RR < 60 mm