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RESEARCH PAPER

Amiloride versus furosemide for the treatment of edema in patients with nephrotic syndrome: A pilot study (AMILOR)

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Abstract

Aim: In rodent models of nephrotic syndrome (NS), edema formation was prevented by blockade of the epithelial sodium channel ENaC with amiloride. However, apart from case reports, there is no evidence favoring ENaC blockade in patients with NS.

Methods: The monocentric randomized controlled AMILOR study investigated the antiedematous effect of amiloride (starting dose 5 mg/day, max. 15 mg/day) in comparison to standard therapy with the loop diuretic furosemide (40 mg/day, max. 120 mg/day) over 16 days. Overhydration (OH) was measured by bioimpedance spectroscopy (BCM, Fresenius). Depending on the OH response, diuretic dose was adjusted on days 2, 5, 8 and 12, and if necessary, hydrochlorothiazide (HCT) was added from d8 (12.5 mg/day, max. 25 mg/day). The primary endpoint was the decrease in OH on d8. The study was terminated prematurely due to insufficient recruitment and a low statistical power due to a low actual effect size.

Results: Median baseline OH was +26.4 (interquartile range 15.5–35.1)% extracellular water (ECW) in the amiloride arm and + 27.9 (24.1–29.4)% ECW in the furosemide arm and decreased by 1.95 (0.80–6.40) and 5.15 (0.90–8.30)% ECW after 8 days, respectively, and by 10.10 (1.30–14.40) and 7.40 (2.80–10.10)% ECW after 16 days, respectively. OH decrease on d8 and d16 was not significantly different between both arms.

Conclusion: The AMILOR study is the first randomized controlled pilot study suggesting a similar antiedematous effect as furosemide. Further studies are required to better define the role of amiloride in NS (EudraCT 2019-002607-18).

KEYWORDS
amiloride, edema, epithelial sodium channels, furosemide, nephrotic syndrome

1 | INTRODUCTION

Nephrotic syndrome (NS) represents a glomerular injury pattern characterized by increased permeability to plasma proteins and heavy proteinuria exceeding 3.5 g/24 h or 3 g/g creatinine. NS is caused by various primary glomerular diseases such as minimal change disease, focal-segmental glomerulosclerosis (FSGS), membranous nephropathy or systemic diseases secondarily affecting the glomeruli such as diabetes. A hallmark of patients with acute NS is overhydration (OH) and edema formation, leading to weight gain, swellings of the legs and eyelids, anasarca, and occasionally effusions in body cavities. In NS, edema formation is caused by renal sodium and water retention and has been explained by the underfill or overfill theory or a combination thereof.[1,2] Meanwhile, there is considerable evidence stemming from murine models of experimental NS that aberrantly filtered serine proteases resulting in proteasuria mediate sodium retention in NS by proteolytically activating the epithelial sodium channel (ENaC) expressed in the distal tubule.[2,3] This notion is strongly supported by the findings that treatment of nephrotic rodents with either the ENaC blocker amiloride or the serine protease inhibitor aprotinin completely prevents sodium retention and edema formation.[4–8] In contrast, inhibitors of the renin-angiotensin system or the mineralocorticoid receptor have typically less to no effect in NS.[6,9–11]

The OH of nephrotic patients can lead to organ dysfunction and cause serious clinical problems such as hypertension, heart failure and pulmonary congestion, eventually increasing mortality.[12] The loop diuretic furosemide, which blocks the Na-K-2Cl cotransporter (NKCC2) in the thick ascending limb, is considered as standard treatment for nephrotic edema in humans.[13] However, the treatment response to furosemide is often diminished in NS, rendering nephrotic edema difficult to treat. In NS, plasma protein binding of furosemide is reduced, leading to increased non-renal clearance and reduced tubular delivery.[14] On a tubular level, the natriuretic response normalized for urinary excretion of furosemide is reduced in nephrotic patients compared to healthy subjects.[14,15]

Given the preclinical results we hypothesized that ENaC inhibition using amiloride might be a rational approach to treat nephrotic edema as first suggested more than 20 years ago.[8,16,17] Deschenes et al. reported enhanced sodium removal when amiloride was added to furosemide in children with NS.[16,17] So far, there are only few smaller studies on the use of amiloride, mainly focusing on the antihypertensive effect in patients without overt NS.[18–20] In NS, use of ENaC inhibitors such as amiloride or triamterene have been reported in single cases,[21–23] however, data from a randomized controlled trial is missing. We therefore initiated the randomized controlled AMILOR study to investigate the anti-edematous effect of amiloride monotherapy in nephrotic patients in comparison to standard therapy with the loop diuretic furosemide.

2 | RESULTS

2.1 | Characterization of the study cohort

From July 2020 until April 2023, $n = 20$ patients were included in the AMILOR study out of which one patient terminated the study prematurely after day 8, leaving $n = 19$ participants who completed the study (Figure 1B). The baseline characteristics of the patients are shown in Table 1. OH determined by bioimpedance spectroscopy was +5.3 [2.9–7.5] l/1.73 m^2 or +26.4 [15.5–35.1]% of ECW volume in the amiloride arm and +6.3 [4.6–7.1] l/1.73 m^2 and +27.9 [24.1–29.4]% ECW in the furosemide arm. Overall, all baseline parameters were not significantly different across both arms (Table 1).

2.2 | Dosing and urinary excretion of amiloride and furosemide

After starting treatment with 5 mg amiloride and 40 mg furosemide, respectively, subsequent doses were escalated during the study according to efficacy and safety parameters (Figure 2A, Table S2). Amiloride was escalated to maximally 15 mg in 50% of the patients while furosemide was escalated to maximally 120 mg in 60% of the patients until day 8. After 8 days, HCT was added with 12.5 mg in 44% and 50% of the patients of the amiloride and furosemide arm, respectively, and after 12 days HCT was escalated to 25 mg in 33% and 30% of the patients in each arm (Figure 2A). The actual median dose of amiloride and furosemide is shown in Figure 2B.

The median urinary furosemide concentration was 6 (4–10) μg/mL on day 2 and increased to 12 (9–20) μg/mL on day 8 (Figure 2C). Subsequently, there was a slight decrease, most likely due to concomitant treatment with HCT. The urinary excretion in 24 h was 26 (26–28)% of the oral dose (Figure 2D). The median urinary amiloride concentration was 1 (1–2) and 2 (1–4) μg/mL on day 2 and 8, respectively (Figure 2C). On day 16, the concentration remained constant (2 [1–5] μg/mL). The median relative urinary excretion was 39 (31–41)% (Figure 2D).

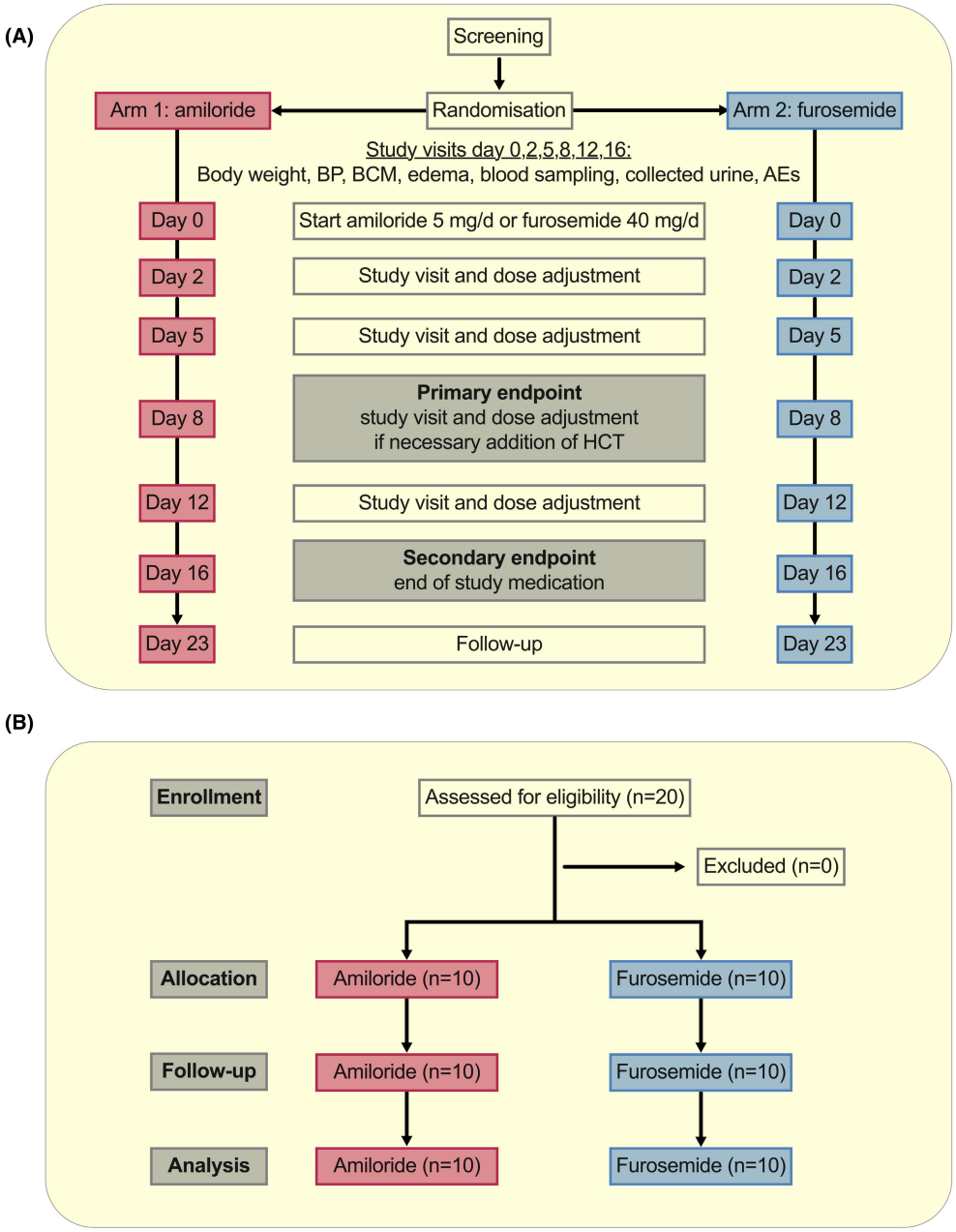

FIGURE 1 Study design (A) and the CONSORT flow chart (B). AE, adverse events; BCM, Body Composition Monitor (Fresenius Medical Care); BP, blood pressure.

2.3 | Treatment effect on the OH and body weight

In both arms, OH decreased as assessed by bioimpedance spectroscopy and normalized for ECW (Figure 3A) or body surface area (Figure 3B). After 8 days, amiloride reduced OH by 1.95 (0.80–6.40)% ECW and furosemide by 5.15 (0.90–8.30)% ECW and on day 16, OH was reduced by 10.10 (1.30–14.40)% ECW in the amiloride arm and by 7.40 (2.80–10.10)% ECW in the furosemide arm, respectively (Figure 3C, Table 2). The difference to baseline was significant for amiloride on day 8 and for both amiloride and furosemide on day 16. Body weight decreased by 3.8 (1.7–7.2) kg in the amiloride arm and by 2.0 (1.5–4.6) kg in the furosemide arm on day 16 (Figure 3D). Both amiloride and furosemide reduced ECW but not intracellular water (ICW, Suppl. Figure S2A,B). The Total body water (TBW) was reduced in parallel to the reduction of ECW while the ratio of ECW to ICW was significantly reduced in both arms on day 16 (Figure S2C,D). Both diuretics had no effect on the course of the hemoglobin concentration or the hematocrit, respectively (Figure S2E,F). No difference for the change of OH (in % ECW) after 8 days

TABLE 1 Characterization of the study groups as well as adverse events, hyperkalemia, and potassium relevant medication.

	Amiloride ($n=10$)	Furosemide ($n=10$)	p-Values
Underlying disease	MCD $n=2$, MN $n=4$, FSGS $n=2$, other $=2$	MCD $n=4$, MN $n=4$, IgAN $n=1$, amyloidosis $n=1$	–
Age (years)	53 (46–51)	50 (25–58)	0.68
Sex	♂ $n=5$/♀ $n=5$	♂ $n=8$/♀ $n=2$	0.35
eGFR (mL/min/1.73 m^2)	69.3 (49.0–91.0)	90.9 (68.3–91.0)	0.06
Proteinuria (g/24 h)	6.5 (5.1–11.7) ($n=8$)	8.7 (7.8–11.6) ($n=9$)	0.08
Albuminuria (g/24 h)	5.5 (4.3–8.8) ($n=8$)	7.8 (7.2–9.0) ($n=9$)	0.23
Plasma albumin (g/24 h)	2.1 (2.0–2.4)	1.7 (1.5–2.0)	0.11
Natriuresis (mmol/24 h)	109 (39–154) ($n=8$)	117 (106–205) ($n=9$)	0.33
OH (%ECW)	+26.4 (15.5–35.1)	+27.9 (24.1–29.4)	0.99
OH (l/1.73 m^2)	+5.3 (2.9–7.5)	+6.3 (4.6–7.1)	0.63
Severe adverse events (SAE)	$n=1$ • delayed discharge from hospital due to macrohematuria after kidney biopsy	$n=5$ • myocardial infarction • abdominal pain and • abdominal pain with diarrhea (2 episodes) with in-hospital treatment in a patient with Crohn's disease • AKI stage 2 with in-hospital treatment • pericardial effusion with in-hospital monitoring	–
Study termination	$n=1$ (after d8) due to treatment refractory edema and impossibility for a dose increase due to hyperkalemia	–	–
Hyperkalemia >5.3 mmol/L	$n=3$	–	–
Potassium-related medication	$n=5$; binder (patiromer 8.4 g OD)	$n=1$; substitution (26–40 mmol OD)	–

Note: Values are median (interquartile range) or number (percent). p-Values are obtained from Mann–Whitney tests; n.s. = not significant.

Abbreviations: ECW, extracellular water (measured by bioimpedance spectroscopy); eGFR, glomerular filtration rate estimated by CKD-EPI_{crea} formula; FSGS, focal-segmental glomerulosclerosis; IgAN, IgA-Nephritis; MCD, minimal change disease, MN, membranous nephropathy; OD, once daily; OH, overhydration (measured by bioimpedance spectroscopy).

was detected between the study groups amiloride and furosemide (primary endpoint, $p=0.380$ one-sided t-test, Table 2), calculated effect size = 0.1388 (95%-CI −0.7446–1.0104). The decrease in OH on day 16 as well as decrease in body weight on day 8 and 16 were also not significantly different between both arms (secondary endpoints, Table 2).

2.4 | Adverse effects

There was one severe adverse event (SAE) in the amiloride arm and five in the furosemide arm, all of them unrelated to the drug treatment (Table 1). All SAEs completely resolved without sequelae. The study was prematurely terminated in one patient of the amiloride arm due to persistent massive edema as the dose could not be increased and amiloride had to be paused due to hyperkalemia (maximum 5.3 mmol/L, Table 1). This led to an amendment to the study protocol with adjustment of potassium thresholds for dose adjustments (valid for all 16 subsequent patients). The respective patient turned out to have a lower actual GFR based on additionally measured Cystatin C concentration (GFR-CKD EPI-CysC 15 mL/min/1.73 m^2). Three patients of the amiloride arm developed hyperkalemia ≥5.3 mmol/L until day 8, five patients received concomitantly the oral potassium binder patiromer. In the furosemide arm, one patient was substituted with oral potassium after day 5 (Table 1).

2.5 | Treatment effect on the blood pressure, eGFR, proteinuria, and urinary serine protease activity

During treatment, systolic and diastolic blood pressure, estimated GFR (CKD-EPI), proteinuria and urinary serine

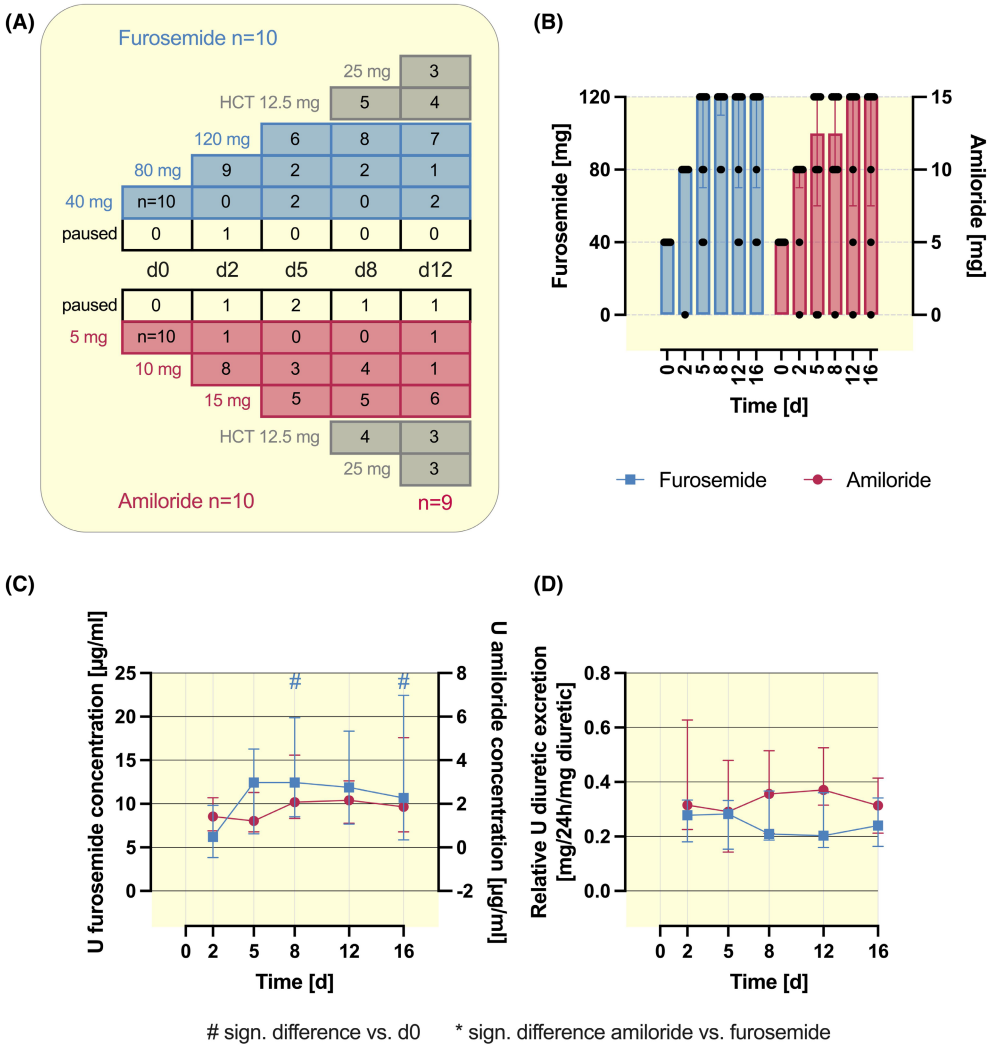

FIGURE 2 Dosing and urinary excretion of amiloride and furosemide. (A) Frequency of the dose used in the study participants as adjusted during the study. (B) Actual median diuretic dose. (C) Urinary concentration in 24 h urine. For conversion in µM, multiply with 0.23 for amiloride and with 0.331 for furosemide, respectively. (D) Urinary excretion in 24 h expressed as proportion of the oral dose. *p*-Values are obtained from Wilcoxon signed-rank tests: $^{\#}p < 0.05$ versus baseline, $^{*}p < 0.05$ between arms.

protease activity against the polybasic tract of γ-ENaC remained constant in both the amiloride and furosemide arm, respectively (Figure 4A–D, Table 2).

2.6 | Treatment effect on sodium and potassium handling

Urine volume increased in both arms to a similar extent (Figure 5A, Table 2) and was paralleled by increased absolute urinary and fractional Na^+ excretion (Figure 5B,C), reaching statistical significance for amiloride at day 8 and for furosemide at day 16. Absolute and fractional urinary potassium tended to be lower in the amiloride arm, however, this did not reach statistical significance (Figure 5D,E). Urinary Na^+/K^+ ratio which reflects ENaC-mediated distal tubular sodium handling tended to increase in the amiloride arm compared to the furosemide arm (Figure 5F).

2.7 | Treatment effect on the plasma Na^+ and K^+ concentration as well as the plasma renin activity and serum aldosterone concentration

Plasma Na^+ concentration slightly decreased in both arms without reaching statistical significance (Figure 6A). As expected, plasma K^+ increased during amiloride treatment whereas it was stable during furosemide treatment (Figure 6B). Plasma renin activity was stimulated during treatment in both arms (Figure 6C). However, serum aldosterone concentration increased solely in the amiloride arm (Figure 6D, Table 2).

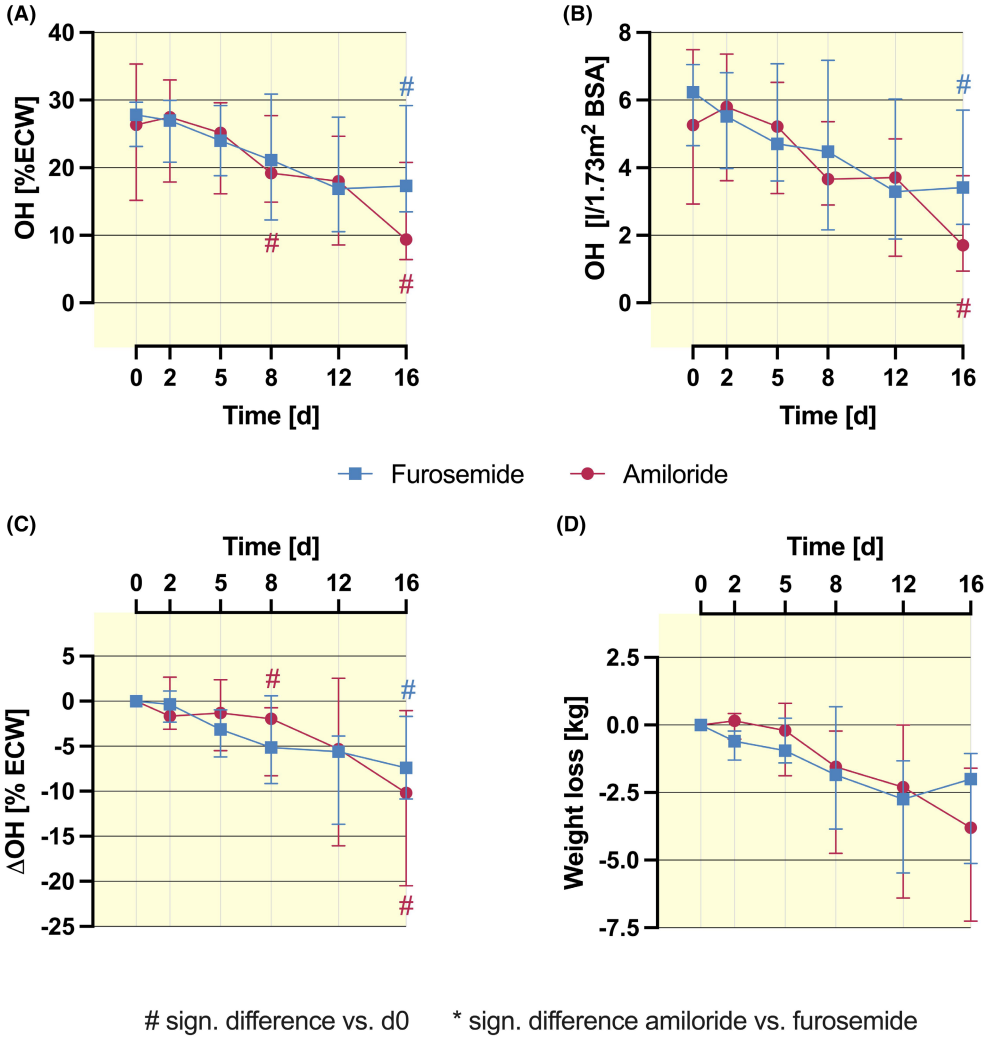

FIGURE 3 Effect of amiloride and furosemide on the OH and body weight. Course of OH as assessed by bioimpedance spectroscopy and normalized for extracellular water (ECW, A) or the body surface area (BSA, B). Course of relative change of the OH (C) and body weight (D). *p*-values are obtained from Wilcoxon signed-rank tests: # $p < 0.05$ versus baseline, *$p < 0.05$ between arms.

3 | MATERIALS AND METHODS

3.1 | Study patients

The study cohort included adult patients (≥18 years at the time of signing the informed consent) with acute NS and proteinuria >3 g/day, who consecutively presented to the nephrology clinic of the University hospital of Tübingen from July 2020 until April 2023 with edema that warranted diuretic treatment. Exclusion criteria included an estimated GFR (CKD-EPI$_{crea}$) <30 mL/min/1.73 m^2, acute kidney injury KDIGO stage 2 or 3, systolic blood pressure <90 mmHg, hyperkalemia (>4.8 mmol/L), signs of cardiac decompensation (orthopnea, dyspnea NYHA IV), current treatment with potassium-sparing diuretics (e.g., spironolactone) or potassium supplements. A detailed list of all inclusion and exclusion criteria is given in Table S1. Study participants required not to have any other diuretic treatment at least 48 h before enrollment. $N = 3$ patients had been treated with torasemide which was stopped more than 48 h before enrollment.

Prior to study entry, a written informed consent was obtained from all patients. The study was conducted in accordance with GCP regulations and the declaration of Helsinki. The study was approved by the local ethics committee of the University of Tübingen (811/2019AMG1) and the BfARM (Federal Institute for Drugs and Medical Devices, 61-3910-4043864). The study was registered at the EudraCT (2019-002607-18) and ClinicalTrials.gov (NCT05079789). The study was monitored by the Center of Clinical Studies of the

TABLE 2 Treatment effects of amiloride versus furosemide (primary and secondary endpoints).

Parameter	*N*	Median with IQR	
Change of OH [%ECW] after 8 days			
Amiloride	10	−1.95 (−6.40 to −0.80)	$p=0.380$, one-sided *t*-test
Furosemide	10	−5.15 (−8.30 to −0.90)	
Change of OH [%ECW] after 16 days			
Amiloride	9	−10.10 (−14.40 to −1.30)	$p=0.358$, two-sided *t*-test
Furosemide	10	−7.40 (−10.10 to −2.80)	
Change of body weight [kg] after 8 days			
Amiloride	10	−1.55 (−4.60 to −0.70)	$p=0.546$, two-sided *t*-test
Furosemide	10	−1.85 (−3.70 to 0.00)	
Change of body weight [kg] after 16 days			
Amiloride	9	−3.80 (−7.20 to −1.70)	$p=0.667$, two-sided *t*-test
Furosemide	10	−2.00 (−4.60 to −1.50)	
Change of systolic blood pressure [mm Hg] after 8 days			
Amiloride	10	−10.00 (−14.00 to −1.00)	$p=0.766$, two-sided Wilcoxon signed-rank test
Furosemide	10	−4.00 (−24.00 to +5.00)	
Change of systolic blood pressure [mm Hg] after 16 days			
Amiloride	9	−15.00 (−22.00 to +2.00)	$p=0.840$, two-sided Wilcoxon signed-rank test
Furosemide	10	−12.50 (−15.00 to −6.00)	
Change of urine volume [mL/24 h] after 8 days			
Amiloride	8	+375 (+100 to +698)	$p=0.221$, two-sided Wilcoxon signed-rank test
Furosemide	9	+150 (−300 to +694)	
Change of urine volume [mL//24 h] after 16 days			
Amiloride	7	+300 (+21 to +600)	$p=0.994$, two-sided *t*-test
Furosemide	9	+200 (0 to +780)	
Change of natriuresis [mmol/24 h] after 8 days			
Amiloride	10	+3.00 (−1.00 to +6.00)	$p=0.559$, two-sided *t*-test
Furosemide	9	0.00 (−1.00 to +5.00)	
Change of natriuresis [mmol/24 h] after 16 days			
Amiloride	9	+2.00 (+1.00 to +5.00)	$p=0.818$, two-sided *t*-test
Furosemide	9	+1.00 (−1.00 to +6.00)	
Change of edema circumference [cm] after 16 days			
Amiloride	9	−3.00 (−4.00 to −1.00)	$p=0.102$, two-sided *t*-test
Furosemide	10	−1.50 (−2.50 to +1.00)	
Change of plasma renin activity [ng Ang/mL/h] after 16 days			
Amiloride	8	+4.85 (+0.90 to +17.55)	$p=0.153$, two-sided Wilcoxon signed-rank test
Furosemide	10	+0.85 (+0.40 to +1.60)	
Change of serum aldosterone concentration [pg/mL] after 16 days			
Amiloride	9	+186.0 (+53.0 to +270.0)	$p=0.008$, two-sided Wilcoxon signed-rank test
Furosemide	10	+8.5 (−10.0 to +16.0)	

Note: Positive values are increases from baseline to day 8 or 16, negative values are decreases, respectively.

University Hospital Tübingen. No major protocol deviation occurred during the study.

3.2 | Study design and the treatment regimen

Patients with NS were randomized to treatment with amiloride (Modamide®, starting dose 5 mg/day, maximal dose 15 mg/day) or furosemide (generic, starting dose 40 mg/day, max. 120 mg/day) over 16 days, followed by an observation period of another 7 days (Figure 1A). Blinding was waived since the drug was easily discernible from the change of the plasma potassium concentration. The OH was measured by bioimpedance spectroscopy (Body Composition Monitor, Fresenius) and determined dose adjustments on days 2, 5, 8 and 12. In the case of insufficient improvement of OH, hydrochlorothiazide (HCT) was added at d8 or d12 (generic, starting dose 12.5 mg/day, max. 25 mg/day). Safety parameters relevant for dose adjustments were plasma creatinine and plasma potassium concentrations. The exact rules for dose adjustments as defined by the study protocol are listed in Table S2. Treatment was done on an outpatient basis except for $n=4$ patients who were included during hospitalization because of NS.

Concomitant medication relevant for the study outcome as defined in the study protocol included ACE inhibitors or angiotensin receptor blockers as antiproteinuric treatment and corticosteroids and other immunosuppressive drugs as therapy of the underlying disease and are reported in Table S3. Adequate prophylactic anticoagulation was given according to standard care. Kidney biopsy to determine the cause of NS was performed after study enrollment in $n=10$ patients after edema and blood pressure control if necessary. Participants were loosely advised to refrain from potassium-rich diets when randomized to the amiloride arm and to potassium-rich diet when randomized to the furosemide arm. A list of some potassium-rich foods was provided to the patients after randomization. In addition, we advised the patients to refrain from excessive fluid intake >2.5 L/day.

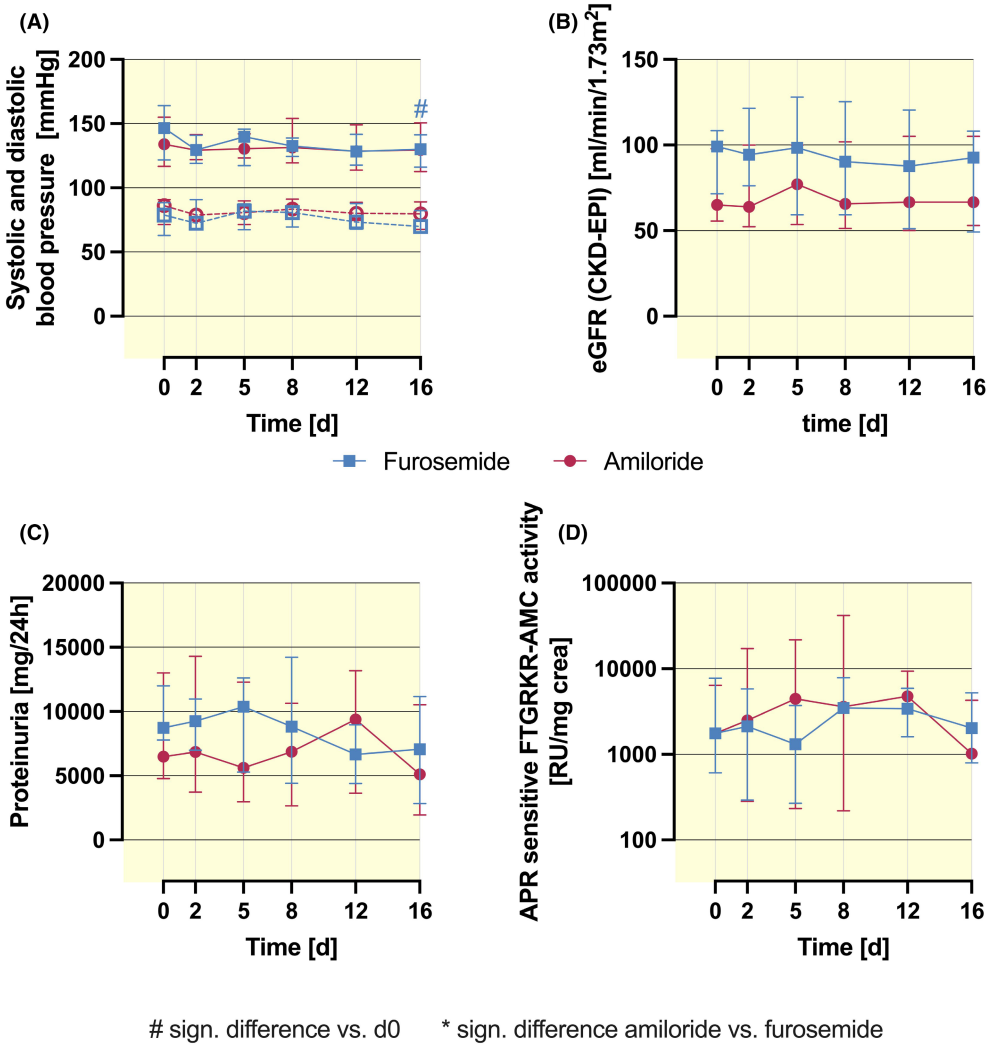

FIGURE 4 Effect of amiloride and furosemide on the blood pressure (A), eGFR (B), proteinuria (C), and urinary serine protease activity (D). Values from 24 h collection urine. Urinary serine protease activity is expressed as aprotinin (APR)-sensitive fraction of the activity against the peptide substrate FTGRKR-AMC, representing the polybasic tract of γ-ENaC. *p*-values are obtained from Wilcoxon signed-rank tests: $^{\#}p < 0.05$ versus baseline, $^{*}p < 0.05$ between arms.

3.3 | Assessment of the fluid status and blood pressure

Fluid status represented by the intracellular water (ICW), extracellular water (ECW), and total body water (TBW) was measured at every study visit using bioimpedance spectroscopy using the Body Composition Monitor (BCM, Fresenius Medical Care). Excess fluid was quantified as so-called OH out of normally hydrated lean and adipose tissue masses. Reference values for OH in healthy individuals lie between −1 and +1L.[29] The values obtained for OH, ECW, ICW, and TBW were normalized to a body surface area of 1.73 m^2. Blood pressure was measured using a calibrated electric blood pressure monitor (Omron, Hoofddorp, the Netherlands) with upper arm cuff as office blood pressure in a sitting position after at least 5 min of rest at the patient's dominant side twice and averaged.

3.4 | Biochemical analyses

Blood and 24 h urine samples were obtained at every study visit. Different parameters were measured as described in the Supplementary Methods section.

3.5 | End points and sample size calculation

Please refer to the Supplementary Material for further details. Briefly, the primary endpoint was the decrease of OH on d8 compared to baseline, expressed as percent of extracellular water (% ECW) to ensure inter-individual comparability. Secondary endpoints were decrease of OH and body weight after 16 days, systolic and diastolic blood pressure as well as edema circumference, increase of

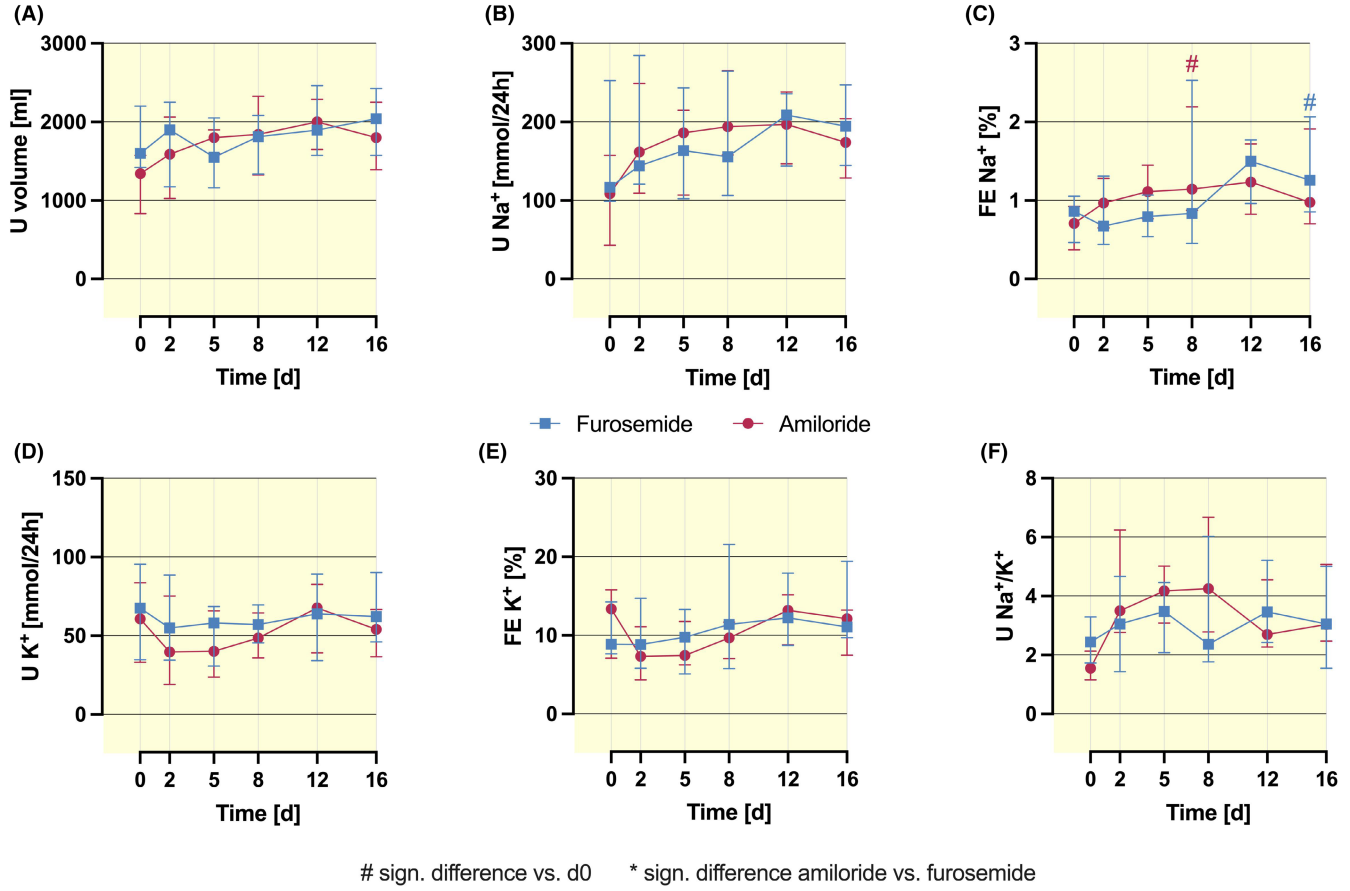

FIGURE 5 Effect of amiloride and furosemide on sodium and potassium handling. (A) Course of urine volume during the study. (B–E) Course of the absolute and fractional urinary excretion of Na^+ and K^+ in 24 h urine. (F) Course of the urinary Na^+/K^+ ratio as a measure of ENaC-mediated distal tubular sodium handling. *p*-Values are obtained from Wilcoxon signed-rank tests: $^{\#}p < 0.05$ versus baseline, $^{*}p < 0.05$ between arms.

urine volume and natriuresis after 8 and 16 days, plasma renin activity and serum aldosterone concentration after 8 and 16 days, number of required changes of dose of study medication, need for co-medication with HCT after 8 days and occurrence of adverse events.

Calculation of sample size yielded a sample size of $n = 18$ patients per group (total $n = 36$; calculated for a *t*-test with the nQuery® Advisor 7.0 program) to prove a superior effect of amiloride over furosemide. Taking dropouts into account, the sample size was defined as $n = 22$ per group (total $n = 44$). The study was not designed to demonstrate non-inferiority, which usually requires a much larger number of participants.

The study was terminated after 34 months prematurely due to insufficient recruitment owing to the SARS-CoV2 pandemic and a lower actual statistical power due to a lower actual effect size than assumed.

3.6 | Statistical analyses

Please refer to the Supplementary Material section for further details. Briefly, all statistical analyses were based on the Intention-to-Treat Population. Primary and secondary endpoints were analyzed by the Institute for Clinical Epidemiology and Applied Biometry of the University Hospital Tübingen. 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. Hereby, the null hypothesis was that there is equal or greater decrease of OH (measured as % ECW) after 8 days in the group of patients with furosemide treatment compared to the group of patients with amiloride treatment. The alternative hypothesis was 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. Safety was assessed by frequency tabulations and line listings for AEs and SAEs.

4 | DISCUSSION

The AMILOR study suggests an antiedematous effect of the ENaC blocker amiloride in patients with NS. Both amiloride and furosemide reduced OH without a statistically

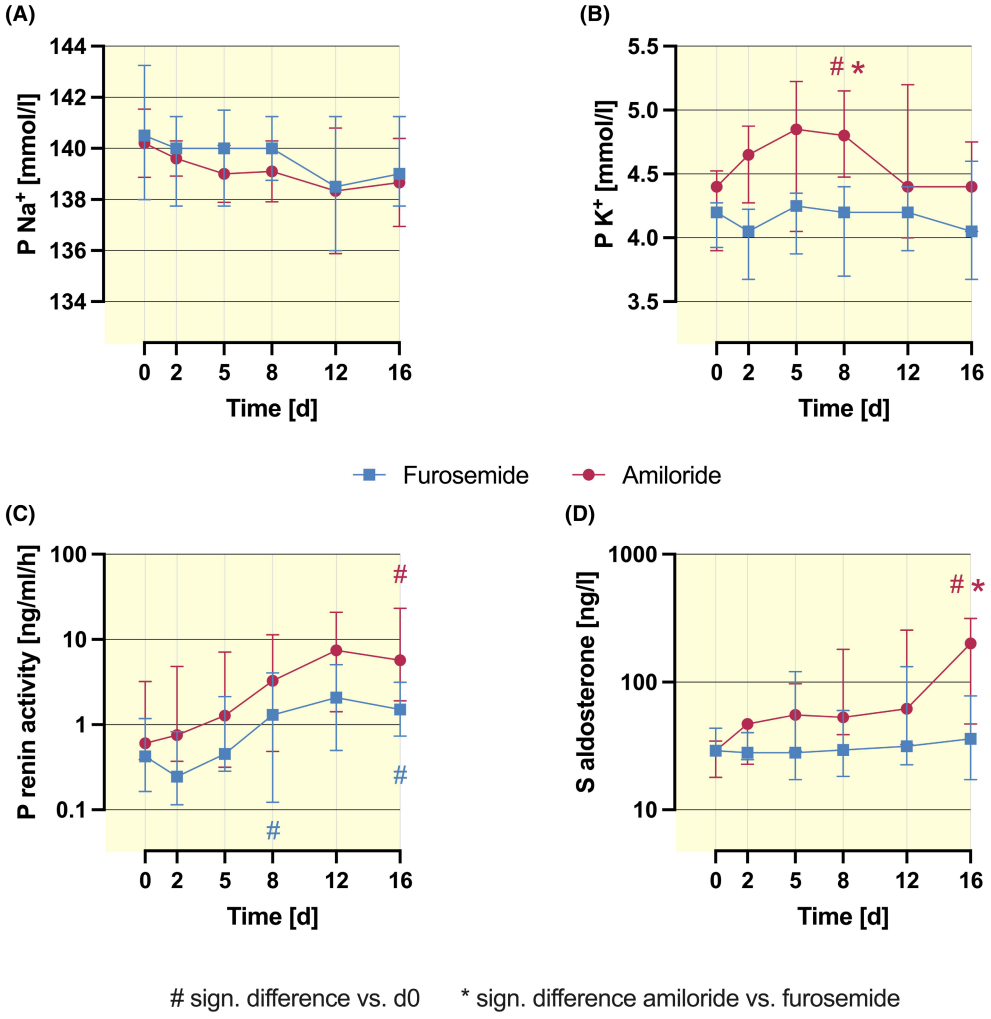

FIGURE 6 Effect of amiloride and furosemide on the course of plasma Na^+ (A) and K^+ (B) concentration as well as the plasma renin activity (C) and serum aldosterone concentration (D). *p*-Values are obtained from Wilcoxon signed-rank tests: $^{\#}p < 0.05$ versus baseline, $^{*}p < 0.05$ between arms.

significant difference between both arms. Diuretic treatments were started at a low dose with adjustments made according to the treatment response and safety signals represented by the plasma sodium, potassium and creatinine concentration. This strategy ensured a very low incidence of adverse effects and treatment withdrawals (only one in the amiloride arm). As expected, amiloride increased and furosemide decreased plasma potassium concentration. In five patients in the amiloride arm, a potassium binder was commenced, in one patient of the furosemide arm potassium was substituted. This indicates that effective doses of each drug were achieved in both arms. The median urinary concentration of amiloride in all samples (2 μg/mL or 7 μM) was markedly above the half-maximal inhibitory concentration (IC_{50}) for ENaC (0.1 μM).[24] In contrast, the median urinary concentration of furosemide in all samples (13 μg/mL or 4 μM) was below the IC_{50} for inhibiting NKCC2 (7 μM).[25] However, due to the process of urine concentration in the collecting duct it is difficult to extrapolate the effective concentrations of the diuretics at their respective transporters in the Henle loop (NKCC2) and distal nephron (ENaC). Yet, it seems that the maximal amiloride dose (15 mg) was reached in this study whereas this was not the case for furosemide (120 mg), which under certain circumstances can be dosed as high as 500 mg/day. Still, the results indicate that both diuretics were equally effective at the doses used in nephrotic patients.

ENaC inhibition with amiloride has been shown to prevent edema formation in three different rodent models of experimental NS,[4,7,8] providing the scientific rationale and motivation for this randomized controlled trial that aimed to translate these findings to patients with NS. The present results provide the first clinical evidence on the efficacy of amiloride monotherapy for the treatment of nephrotic patients which has been long awaited and formulated in the 2021 KDIGO guidelines on glomerular diseases.[13] The natriuretic effect of amiloride in NS supports the speculation that ENaC might also be activated in human NS and the

site of sodium retention without stimulation of the renin-angiotensin-aldosterone system (Figure 6).

A notorious and potentially life-threatening side effect of amiloride is the promotion of hyperkalemia, owing to the essential role of ENaC in maintaining potassium homeostasis. The hyperkalemic potential of amiloride is at least as high as that of mineralocorticoid receptor antagonists (MRAs). The susceptibility to develop hyperkalemia upon ENaC inhibition is increased at a lower GFR and with a higher amiloride dose. In the study of Unruh et al., a dosage of 20 mg of amiloride given over 14 weeks was associated with hyperkalemia and acute deterioration of renal function even in the absence of reduced GFR before treatment.[19] Once GFR is reduced e.g. by a potent diuretic effect, amiloride further accumulates due to prolongation of its clearance,[26] initiating a vicious cycle. Therefore, amiloride should not be given in higher doses and not used in patients with reduced GFR. In the one patient terminating the study in the amiloride arm, initial GFR was overestimated and turned out to be <30 mL/min/1.73 m^2 as estimated by the plasma cystatin C concentration. Conversely, in young patients with NS and preserved GFR, amiloride is potent and safe, particularly when the dose does not exceed 10–15 mg/day. In any case, nephrotic patients under ENaC inhibition must undergo regular checks of plasma potassium concentration. In addition, hyperkalemia risk can be mitigated by the use of potassium binders.

So far, there have been two randomized controlled trials that have tested diuretic treatments in adult patients with NS. In the study by Fallahzadeh et al.,[27] patients of group 1 ($n = 10$) received treatment with 250 mg of acetazolamide and 50 mg of HCT daily and group 2 ($n = 10$) received 40 mg of furosemide and 50 mg of HCT daily during the first week. In the second week, all patients received furosemide (40 mg). After 14 days of treatment, patients of group 1 experienced a slightly higher weight loss, however, the overall effect was modest. The second trial was published recently by Fratila et al.,[28] comparing treatment with intravenous furosemide (160 mg/day given continuously) or a combination of oral furosemide (40 mg, one tablet), amiloride and HCT (one tablet with 5/50 mg). The study enrolled $n = 11$ patients in each arm from which $n = 8$ patients in the i.v. furosemide arm and $n = 10$ patients in the combination arm completed the study and entered the final analysis. Both regimens were very effective and drastically reduced body weight by 5 to 7 kg within 5 days of inpatient treatment. However, this was associated with adverse effects leading to study termination of $n = 3$ patients in the i.v. furosemide arm and one patient in the arm with combination treatment due to hyperkalemia. Although the efficacy of the amiloride-based regimen was remarkable, the study does not allow inferences of a possible ENaC activation in human NS as the effect is confounded by the effects of the other diuretics used.

The AMILOR study is limited by its small study size caused by a failure to recruit the desired number of participants, partially owing to the corona pandemic. The effect of amiloride seemed to be modest and incomplete, but this was also the case for furosemide. This might be related to the study design with a low starting dose and slow uptitration. As can be seen in Figure 3A, the reduction of the OH curve became steeper in the amiloride arm after day 5 indicating that an effective ENaC inhibition was established. Given the slow antiedematous effect of both diuretics a primary end point longer than 8 days from initiation would have led to improved correction of OH. However, this would have interfered with the specific treatment of the underlying disease such as minimal change disease with prednisolone. Overall, the results of this study are only hypothesis-generating with regard to the efficacy of amiloride in NS, however, it provides important clues for the design of a larger study powered to better define the role of amiloride in the treatment of nephrotic edema in comparison to other diuretic regimens or combinations thereof (e.g. furosemide and amiloride).

5 | CONCLUSION

The AMILOR study is a randomized controlled pilot study on the use of the ENaC blocker amiloride in NS suggesting a similar antiedematous effect as furosemide. Thus, amiloride emerges as an alternative to the standard therapy with furosemide. The knowledge gained forms the basis for the design of a larger multicenter study with greater statistical power.

AUTHOR CONTRIBUTIONS

Anja Schork: Conceptualization; data curation; formal analysis; funding acquisition; writing – review and editing; project administration; validation; visualization. **Elisabeth Vogel:** Data curation; writing – review and editing; project administration; validation. **Bernhard N. Bohnert:** Data curation; formal analysis; writing – review and editing; validation; visualization. **Daniel Essigke:** Data curation; writing – review and editing. **Matthias Wörn:** Data curation; writing – review and editing. **Imma Fischer:** Formal analysis; writing – review and editing. **Nils Heyne:** Conceptualization; data curation; writing – review and editing; project administration; supervision. **Andreas L. Birkenfeld:** Writing – review and editing; resources. **Ferruh Artunc:** Conceptualization;

data curation; formal analysis; funding acquisition; writing – original draft; writing – review and editing; project administration; validation; visualization; supervision.

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CONFLICT OF INTEREST STATEMENT

All the authors declared no competing interests.

DATA AVAILABILITY STATEMENT

Original data that support the findings of this study as well as the statistical report are available online. All the material submitted is conform with good publishing practice in physiology.[30]

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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