The MIcrocirculation, dialysis MOdality and sequestered SAlt (the MIMOSA study)

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Coordinating investigator/project leader	dr. M.P.C. Grooteman Amsterdam UMC, location AMC mpc.grooteman@amsterdamumc.nl +31 20 732 09 08
Principal investigators **Per study site**	dr. M.P.C. Grooteman, internist/nephrologist, dr. C.L.M. de Roij van Zuijdewijn, internist/nephrologist, clinical epidemiologist, prof. dr. M.J. Nubé, em. internist/nephrologist Niercentrum aan de Amstel: dr. G.F. van Breda, internist/nephrologist Dianet (location Amsterdam): dr. L. Jakulj, internist/nephrologist
Sponsor	Stichting Amsterdam UMC De Boelelaan 1117 1081 HV Amsterdam The Netherlands
Subsidising parties	Dutch Kidney Foundation (Nierstichting Nederland), Bussum, the Netherlands Niercentrum aan de Amstel, Amstelveen, The Netherlands

	B.Braun Avitum AG, Melsungen, Germany
Independent expert	Drs. J. Kooter, internist Amsterdam UMC jkooter@amsterdamumc.nl 06-25716079
Advisors	Prof. dr L. Vogt and dr R.H.G. Olde Engberink, both internist/nephrologist, Amsterdam UMC
Laboratory sites	1. Laboratory Experimental Clinical Chemistry, Amsterdam UMC, location AMC, Meibergdreef 9, Amsterdam, the Netherlands. dr. R. Nieuwland, Biologist 2. Laboratory for General Clinical Chemistry, Amsterdam UMC, location AMC, Meibergdreef 9, the Netherlands, dr. H.J. Bontkes, Medical Immunologist 3. Laboratory of Genetic Metabolic Diseases, Amsterdam UMC, location AMC, Meibergdreef 9, the Netherlands. dr. F. M. Vaz, Clinical Biochemist

PROTOCOL SIGNATURE SHEET

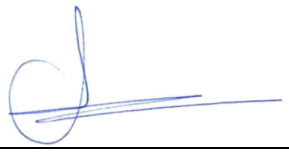
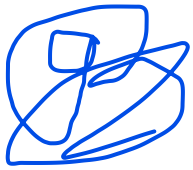
Name	Signature	Date
Head of Department: Prof. dr. F.J. van Ittersum, internist/nephrologist, head of division of Nephrology		4-2-2026
Principal Investigator: dr. M.P.C. Grooteman, internist/nephrologist		30-01-2025
Principal Investigator Niercentrum aan de Amstel: Dr. G.F. van Breda, internist/nephrologist		3-2-2026
Principal Investigator Dianet (location Amsterdam): dr. L. Jakulj, internist/nephrologist		03-02-26

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
BIA	**Bio-electrical Impedance Analysis**
BP	**Blood Pressure**
BV	**Blood Volume**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CFU	**Colony Forming Units**
CKD	**Chronic Kidney Disease**
CV	**Cardiovascular**
CVD	**Cardiovascular Disease**
DBP	**Diastolic Blood Pressure**
DNa	**Sodium concentration in dialysis fluid**
DSI	**Dialysis Symptom Index**
DSMB	**Data Safety Monitoring Board**
ECC	**Extra Corporeal Circuit**
ESKD	**End-Stage Kidney Disease**
EU	**Endotoxin units**
EU	**European Union**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
FO	**Fluid Overload**
GAG	**Glycosaminoglycan**
HD	**Hemodialysis**
HDF	**Hemodiafiltration**
hvHDF	**High volume hemodiafiltration**
IDH	**Intradialytic Hypotension**
IB	**Investigator's Brochure**
IC	**Informed Consent**

IMDD	**Investigational Medical Device Dossier**
IPD	**Individual Patient Data**
LVH	**Left ventricular hypertrophy**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
MC	**Microcirculation**
MMW	**Middle Molecular Weight**
MPS	**Mononuclear Phagocyte System**
NO	**Nitric Oxide**
PNa	**Sodium concentration in plasma**
PWV	**Pulse Wave Velocity**
RRF	**Residual Renal Function**
(S)AE	**(Serious) Adverse Event**
SDF	**Side stream Dark Field**
SSC	**Sequestered Sodium Content**
SBP	**Systolic Blood Pressure**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TSB	**Tissue sodium balance**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
UF	**Ultrafiltration**
UP	**Ultrapure**
VEGF-C	**Vascular Endothelial Growth Factor-C**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: The life expectancy of patients with end-stage kidney disease (ESKD) is poor. Recently, a large randomized controlled trial showed that treatment with high-volume post-dilution HDF improves survival when compared to standard HD (1). It is unclear, however, *why* (hv)HDF improves survival. Neither an increased clearance of middle molecular weight uremic toxins, nor improved BI explains the difference. As the effects are already observed within 2.5 years, recovery of a functional disorder is more likely than restoration of structural alterations. Recuperation of vascular dysfunction may be the missing piece of the puzzle. Previous literature showed (a) that the MC is severely disturbed in dialysis patients, (b) excess sodium can bind to glycosaminoglycans - which are present in subcutaneous storage depots and in the glycocalyx of blood vessels - without commensurate water retention (sequestered sodium content [SSC]) and (c) that in patients with non-dialysis dependent chronic kidney disease (CKD), a disturbed SSC is related to capillary rarefaction and dysfunction. Therefore, in this study we will assess the influences of both the modality (HD and HDF) and the dialysate sodium concentration (DNa) on the SSC and the MC.
Objective: five hypotheses are evaluated: (1, 2) treatment with hvHDF improves the SSC and MC, when compared to HD; (3, 4) disorders of the SSC and the MC are influenced by differences between the dialysate sodium [DNa] and plasma sodium [PNa] concentrations; (5) SSC and MC are interrelated in this patient group.
Study design: randomized cross-over trial.
Study population: Prevalent adult ESKD patients (≥18years), treated with HD or HDF.
Intervention: participants will be subjected to 5 treatment modes in random order:
1. HD with expected diffusive sodium balance: DNa=PNa; 2. HD with expected diffusive sodium efflux: DNa<PNa, difference -3 mmol/L; 3. HD after isolated ultrafiltration (UF): DNa=PNa; 4. hvHDF with expected diffusive sodium balance: DNa=PNa; 5. hvHDF with expected diffusive sodium efflux: DNa<PNa, difference -3 mmol/L.
Main study endpoint: SSC in muscle as measured by ^{23}Na-MRI. Main secondary endpoints: skin SCC and MC as measured by skin perfusion/vasoreactivity.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Only established dialysis modalities are performed. Yet, while a low DNa has been associated with a lower UF need, intradialytic hypotension (IDH) may occur more often. Five MRIs will be performed in 20 weeks to assess the SSC (before dialysis, Spinoza Centre). In 5 patients, this will be done both before and after dialysis. MC-function will be assessed non-invasively by iontopheresis [acethylcholine and sodium nitroprusside]. Eleven times blood will be drawn from the ECC (no venepuncture). Finally, participants will be asked to fill in 3 questionnaires 5 times.

1. INTRODUCTION AND RATIONALE

1.1 Abstract

From a recent meta-analysis of four randomized controlled trails (RCT), it appeared that mortality in HDF is lower than in HD, especially when high convection volumes are achieved (high-volume HDF; hvHDF) (2). Curiously, this effect was already manifest after just 2.5 years in an elderly high-risk population. Recently, a large multinational, RCT (CONVINCE) comparing hvHDF with high-flux HD in 1360 patients confirmed these findings (1). As it is unlikely that structural changes are restored in this short time period, hvHDF may repair a *functional* defect with a cumulative benefit due to the repetitive character of the treatment. In theory, three main mechanisms may be involved: 1) enhanced removal of middle molecular weight uremic toxins, 2) improved BI, 3) low IDH-frequency and hence a less disturbed tissue perfusion (3). Recent observations indeed show that IDH occurs less often during hvHDF than during HD. Whether these macrovascular effects are accompanied or driven by microcirculatory (MC) improvements – possibly by a restored sequestered sodium content (SSC) homeostasis – is currently unclear. As the SSC encompasses water-free Na^+, its reduction may occur without changes in dry weight and BP. Therefore, we hypothesize that (1 and 2) the SSC and the MC are better preserved in hvHDF than in HD, (3) a lower DNa leads to a reduced loading of the SSC and (4) the SSC and MC are interrelated.

1.2. End-stage kidney disease (ESKD) and premature cardiovascular (CV) disease

Besides fluid overload (FO, (4, 5), ESKD patients generally suffer from oxidative stress, inflammation and endothelial dysfunction. Together with chronic kidney disease – mineral and bone disorder (CKD-MBD), traditional CV risk factors and anaemia, premature CV-disease arises. Besides large vessel disease, MC defects are also often present (6, 7).

1.3. Hemodialysis, intra-dialytic hemodynamic instability and organ perfusion

Although HD-treatment prolongs life, serious drawbacks may occur, e.g. due to the BI of the extra-corporeal circuit [ECC, (8)] and IDH, which - depending on its definition – occurs in 10-25% of the dialysis sessions and is related with mortality when systolic BP (SBP) declines to <90 mmHg or <100 mmHg with a pre-SBP of <160mmHg and ≥160mmHg, resp. (9). Yet, a stable BP may not be a sensitive marker for blood flow in vital organs, exemplified by proven cardiac hypoperfusion already after 30 minutes, when dry weight and BP were unchanged (10). Notably, sublingual blood flow did not decrease *during* HD, but afterwards. Thus, MC-perfusion changes occur independently in different tissues. Moreover, variable relations existed between FO, BP and perfusion deficits in the heart (10-12) and brain (13).

1.4. Salt and water overload, non-osmotic Na+ storage and the microcirculation

In ESKD, often an imbalance exists between the intake and removal of salt and water. Most patients suffer from chronic FO (5). (4, 14).Several studies showed an association between chronic FO (>15% ≈ 2.5L) and fatal CVD (5, 15, 16). At the same time, a high UF-rate (>10 ml/kg/min) is related to mortality (17).

Both animal and human studies showed that Na+ can be stored without commensurate water retention (18). These depots operate independent of renal function, and when perturbed, react inadequately upon salt loading (19). In rats on a high-salt diet, precapillary skin vessels showed increased vasoreactivity, resulting in increased peripheral vascular resistance (20). As skin and muscles receive 25% of cardiac output, these alterations could have a major impact on BP (21). In ESKD patients, a high SSC was associated with capillary rarefaction, increased vessel permeability (22) and reduced NO release (6). In combination with precapillary calcifications (23), these alterations may contribute to MC dysfunction. In CKD, SSC correlated with left ventricular hypertrophy (LVH), independent of BP and FO (24).

1.5. HDF improves survival most likely by a correction of functional abnormalities

HDF prolongs life by reducing *cardiac* mortality in particular (2, 25). As such, a low IDH-incidence may play an important role (3). Yet, given the magnitude of the survival gain, it is questionable whether the low IDH-frequency only explains the outcome. The rate at which HDF improves survival in prevalent ESKD patients, who are generally characterized by chronic FO (26), widespread CVD (27), and a CKD-history >5 years, is astonishing. As the improved survival is already observed within 2.5 years (1), it is more likely that a *functional condition* is corrected by HDF rather than a *structural disorder*.

1.6. Water-free tissue sodium is a potential reversible uremic toxin in ESKD

Sodium stimulates thirst and FO in ESKD patients. Vice versa, strict salt control is associated with improvements in LVH, IDH and survival (28, 29). According to the classic view (30), salt and water are located in the extracellular compartment, which consists of the interstitial space and vascular system, manifesting itself as edema with or without hypertension when present in excessive amounts. Yet, on top of that, non-osmotic tissue sodium has been demonstrated. High SSC values can be found in a variety of conditions, e. g. aging, male gender, salt sensitive hypertension (31), LVH, (24) and advanced CKD (5, 32).The SSC was normalized by HD treatment in patients >60 years, whereas in those <60 years it was reduced below controls (33). So, first it appears that sodium in HD patients is iso-osmotic bound to water in the extracellular compartment and water-free in storage depots

(34). Second, both water-bound and water-free sodium are removed by HD, which occurs independent of each other (33). As the SSC can be lowered by furosemide and HD (35), these depots are dynamic and may function as buffers for the *uptake, storage and release of excess sodium* (36).

Besides dietary measures, reduction of dialysate sodium (DNa) may help to avoid sodium accumulation. When DNa exceeds the plasma concentration (PNa), sodium enters the body and induces thirst and FO. When PNa exceeds DNa, however, sodium is removed from the circulation, inducing a change in osmolality and a decline in BV. As a result, IDH can arise, which impedes UF and results in a higher post-dialysis weight.

Since 7% of the plasma consists of lipids and proteins, the actual PNa is 100/93 x [blood Na+]. Yet, due to the Gibs-Donnan effect, which refers to the interactions between PNa and negatively charged plasma proteins, a small fraction (5%) is not available for diffusion. Hence, in clinical practice these effects largely compensate each other (37). In s-HD, roughly 80% of sodium is removed by UF and 20% by diffusion (38).

In HDF, a surplus of plasma water is extracted from the circulation and replaced by substitution fluid. Here, sodium balance will depend on PNa, the total convection volume (substitution volume plus UF) and the concentration in the substitution fluid, which is similar to DNa. Whereas sodium removal by diffusion and UF is largely comparable between HD and HDF (39), large convection volumes may influence sodium balance, depending on the plasma-dialysate gradient. In HDF with a convection volume of 25 L and a sodium gradient of 3 mmol/L, maximum Na+ flux - in either direction - is 75 mmol. Although sodium removal can be substantial at the patient level, the negative and positive effects of sodium in- and efflux may compensate each other at the group level. Hence, it is unlikely, that a clinical detectable surplus of sodium removal explains the effects of HDF on survival (2). For example, in ESHOL, mean pre-dialysis PNa was 138.8 mmol/L and DNa 138-140 mmol/L (40). In the Turkish HDF study these values were 136 ± 3 mmol/L and 138 mmol/L, resp. (41). Thus, most likely sodium balance was positive in some and negative in others.

Only a few studies compared sodium removal between HD and H(D)F. Based on a series of equations, Locatelli et al. estimated that sodium removal was similar in hemofiltration (HF), low-flux HD and predilution HDF (42). Previously, a mathematical model was developed, which predicted similar sodium losses in HD and HDF (43). More recently, comparable amounts of sodium removal were reported in HD and HV-HDF (39). In none of these studies, however, the SSC was taken into account. Even so, these findings are compatible with recent data from the IPD cohort (44). As HDF was associated with improved survival, (2) and BP and dry weight decreased similarly over time (3), it is unlikely that differences in iso-osmotic sodium transport underlie this effect. *Yet, it is conceivable that a gradual discharge of the SSC in HDF patients is paralleled by a correction of a functional MC defect.*

1.7. The (patho)physiology of water-free tissue sodium balance in health and disease.

Since water-free tissue sodium is bound to negatively charged glycosaminoglycans (GAG), simple biological kinetics, such as concentration differences or iso-osmotic UF, cannot explain its mobilization during HD. In rats on a high salt diet, skin interstitium - which represents a separate micro-environment - triggers a series of mechanisms that keep its tonicity and volume constant. Skin sodium accumulation induces osmotic stress, which initiates a mononuclear phagocyte system (MPS) mediated activation of the tonicity-responsive enhancer binding protein/vascular endothelial growth factor-C (TONEBP/ VEGF-C) regulatory axis, which in turn induces hyperplasia of the lympho-capillary network and increased eNOS expression in interstitial endothelial cells (31). Thus, tissue specific MPS cells act as local controllers of interstitial volume, BP and electrolyte homeostasis (45).

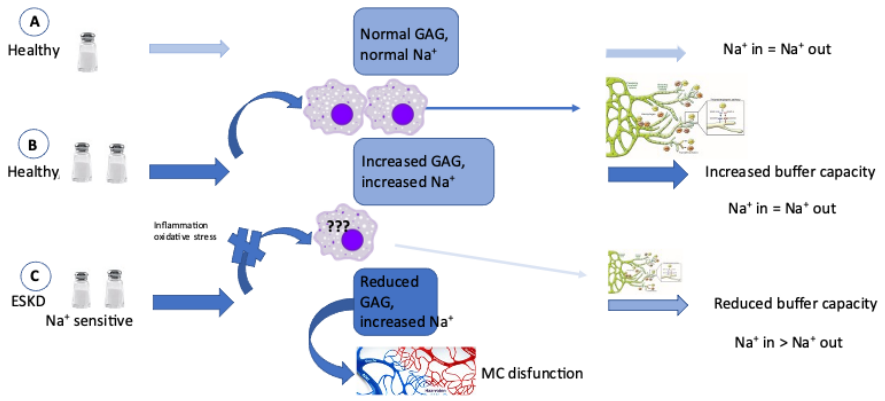


Figure 1: Schematic overview of non-osmotic Na^+ storage in health and disease

In summary: in healthy conditions, excess sodium is iso-osmotic bound to water and reversible water-free to GAGs, both in the glycocalyx of blood vessels (46), and in storage tissues in various organs (34, 47). During osmotic stress, resident MPS cells stimulate lymphangiogenesis, which is vital for sodium-efflux. While a high salt load stimulates GAG formation (34), chronic inflammation inhibits this process. Since a single HD-session reduced SSC to control levels in patients >60 years and below in those <60years (33), it seems justified to conclude that HD treatment abrogates a *functional* blockade of tissue sodium discharge, either via a restored lymphatic drainage or another - unknown, but apparently instantaneous - process, which promotes its direct mobilization and removal. Whether these effects are mediated by the elimination of uremic toxins, reductions in oxidative stress and (micro)inflammation (48), and/or an improvement of endothelial function, is a matter of

speculation. *Yet, considering the beneficial effects on all these aspects (49), HDF might restore the SSC to a greater extent than HD.*

1.8. Summary: Improvement of the water-free Na+ balance by hemodiafiltration

Hemodiafiltration and survival: hvHDF is associated with a better (cardiac) survival than HD, which may depend (in part) on a lower IDH-incidence and less organ damage. Since the survival difference was manifest after only 2.5 years, relief of a functional disorder, such as an improved microcirculation, is most likely.

Salt and fluid overload in HD and HDF: An overload of both sodium and fluid are often present in ESKD. Based on theoretical considerations, clinical studies and a mathematical model, iso-osmotic sodium removal is similar in HD and HDF. Whether a surplus of Na+ is removed in HDF depends on the concentration gradient between DNa and PNa, and the magnitude of the convection volume. At the group level, sodium in- and efflux may have compensated each other in the IPD cohort. Hence, differences in iso-osmotic sodium removal do not explain the survival benefit of HDF. However, none of these studies assessed the effects of dialysis on the SSC, which has been related to the functioning of the microcirculation. Yet, reminding the simple and crude estimations of the cited studies, it cannot be excluded that an improved SSC homeostasis underlies the beneficial effect of HDF on survival (50), possibly mediated by an improved microcirculation. In this study, we will investigate (1, 2) whether the SSC and the MC are better preserved in hvHDF than in HD, (3) whether a lower dialysate sodium content leads to a reduced loading of the SSC and (4) whether the SSC and MC are interrelated in ESKD.

Clinical impact:

The current study may provide evidence that treatment with hvHDF induces a better-preserved MC and SSC homeostasis and that treatment with a lower dialysate sodium improves SSC and MC. Given the previously shown survival benefit of hvHDF, the current study could lead to a preferred dialysis treatment of hvHDF with a diffusive sodium efflux (DNa<PNa). In addition, because intradialytic hemodynamic stability is better preserved in hvHDF, it is conceivable that sodium removal could be better tolerated. Regardless of the outcome, this study will enhance our knowledge about various dialysis treatments and its consequences on SSC and MC and will, therefore, enable us to further optimize dialysis prescriptions for individual cases to ultimately improve both quantity and quality of life of these patients.

2. OBJECTIVES

Primary Objective

To determine the difference in sequestered sodium content (SSC) in muscle tissue after 4 weeks of treatment with:

- HDF with an expected zero diffusive sodium balance (DNa=PNa) and
- HD with an expected zero diffusive sodium balance (DNa=PNa)

Secondary Objectives

To assess and compare the effects of 4 weeks of treatment with the following dialysis modes

- HDF with an expected zero diffusive sodium balance (DNa=PNa);
- HDF with an expected diffusive sodium efflux (DNa < PNa, difference -3 mmol/L);
- HD with an expected zero diffusive sodium balance (DNa = PNa);
- HD with an expected diffusive sodium efflux (DNa < PNa, difference -3 mmol/L), and
- Isolated ultrafiltration for 30 minutes followed by HD with an expected zero diffusive sodium balance (DNa = PNa)

on the following outcomes:

- The SSC in muscle and skin
- The microcirculation
- The SSC and MC in a single treatment session
- Symptoms during treatment
- Thirst and interdialytic weight gain
- Frequency of intradialytic hypotension
- Interdialytic blood pressure
- Markers of organ damage and tissue injury
- Markers of inflammation
- Markers of endothelial damage
- Markers of the SSC

3. STUDY DESIGN

Randomized cross-over trial in prevalent dialysis patients, comparing 5 different combinations of dialysis mode and dialysis fluid sodium content:

1. HD with expected diffusive zero sodium balance (DNa = PNa);
2. HD with expected diffusive sodium efflux (DNa<PNa, difference=3 mmol/L);
3. HD after isolated ultrafiltration (DNa=PNa);
4. hvHDF with expected diffusive zero sodium balance (DNa=PNa);
5. hvHDF with expected diffusive sodium efflux (DNa<PNa, difference=3 mmol/L)

To assess the cumulative effect of 4 weeks of treatment with a certain combination of dialysis mode and dialysis fluid sodium content on the SSC and the MC, sodium-MRI and MC measurements will be performed before the last dialysis session in this particular (randomized) order, as shown in figure 1. In 5 patients, MC and MRI measurements will be carried out both before and after the last dialysis session in order to, additionally, investigate the acute effects of one single dialysis treatment on the MC and the SSC.

	Baseline			*Study phase*																											
Week	0			1-3			4			5-7			8			9-11			12			13-15			16			17-19			20		
Modality	Regular			HDF			HDF			HDF			HDF			HD with iUF			HD with iUF			HD			HD			HD			HD		
Na⁺	Regular			DNa=PNa			DNa=PNa			DNa<PNa			DNa<PNa			DNa=PNa			DNa=PNa			DNa=PNa			DNa=PNa			DNa<PNa			DNa<PNa		
Treatment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MRI (pre)									X						X						X						X						X
MC (pre)									X						X						X						X						X
MRI* (post)									X						X						X						X						X
MC* (post)									X						X						X						X						X
Lab			X						X						X						X						X						X
BP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PRO									X						X						X						X						X

Figure 1: schematic overview of the study. Treatment order will be randomized.

Abbreviations: Na+ = sodium, HDF = hemodiafiltration, HD = hemodialysis, DNa = dialysate sodium, PNa = plasma sodium, MRI = magnetic resonance imaging, MC = microcirculation, lab = laboratory assessments, BP = blood pressure, PRO = patient-related outcomes

* These measurements will be performed in 5 patients

4. STUDY POPULATION

4.1 Population (base)

Patients treated chronically (≥3 months) with intermittent HD or HDF are eligible for participation in the present study. They will be recruited from Niercentrum aan de Amstel (50 prevalent dialysis patients), Amstelveen, The Netherlands, and from Dianet (about 160 prevalent dialysis patients), Amsterdam, the Netherlands.

4.2 Inclusion criteria

- Age ≥18 years
- Treatment with an HD or HDF 3 x per week during at least 4 hours for at least 3 months
- Blood flow rate ≥350 ml/min
- Residual diuresis <200 ml/day
- Plasma Na before dialysis 137-145 mmol/L
- spKt/Vurea ≥ 1.2
- Ability to understand study procedures
- Willingness to provide informed consent

4.3 Exclusion criteria

- Severe incompliance to dialysis procedure and accompanying prescriptions, especially frequency and duration of dialysis treatment
- Life expectancy < 3 months due to non-renal disease
- Expected transplantation within 6 months
- Access recirculation > 10%
- Participation in another clinical intervention trial
- Metal implants (e.g., implantable cardioverter defibrillators)
- Severe obesity (as MRI diameter is 60 cm) and/or calf circumference < 38 cm
- Claustrophobia

4.4 Sample size calculation

The power calculation applied is based on a cross-over study design with a two-tailed $\alpha=0.05$, $\beta=0.80$ and an expected change in the pre-dialysis muscle sodium concentration of 1 mmol/L within 4 weeks, with an estimated standard deviation of 3.0 mmol/L for this change, and a within subject correlation coefficient of 0.9. Using G*power with a matched pairs t-test, the required sample size was calculated to be 17 participants. Given the study's expected burden, a 25% dropout rate is accounted for, resulting in a tar get sample size of 23 participants.

As our primary aim is to determine whether improved SSC homeostasis might underly the survival benefit of HDF, a matched pairs t-test for two dependent means was chosen to compare HDF and HD, both with a zero sodium balance (DNa = PNa).

Currently, there is no literature available on the within-subject variability of the sequestered salt content between repeated measurements in dialysis patients. However, we anticipate a high correlation between repeated measurements within a subject, as tissue sodium content is largely dependent on stable patient characteristics, such as age, gender, hypertension and renal failure. We assume that pre-dialysis values of subcutaneous sodium storage will remain relatively stable over a 4-week period, providing the dialysis modality and dialysate sodium concentration do not change. Therefore, we applied a correlation coefficient of 0.9. The standard deviation of 3.0 mmol/L is based on the results from the study by Dahlmann et al (33). In this study, the average standard deviation was 3.35 mmol/L, calculated as a pooled mean of the standard deviations of the pre-dialytic muscle tissue sodium concentrations in participants <60 years (SD 2.6 mmol/L, n=10) and ≥60 years (SD 4.1 mmol/L, n=14), respectively. Since this study populationwas a heterogeneous group with varying residual kidney function - where natriuresis could influence tissue sodium concentration - we expect a smaller variability in our population (only patients with residual diuresis < 200 ml/day).
The expected drop-out rate of 25% is based on a previous study (51), with a dropout rate of 11%. In the present study, this percentage is anticipated to be higher due to the increased burden (although in the present study, participants will receive compensation for their study-related time investment (see section 11.6 ‘Incentives’).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Dialysis fluid: All treatments will be performed with ultrapure (UP) dialysis fluids (< 0.1 CFU (colony forming units)/ml, <0.03 EU (endotoxin units)/ml. Dialysate flow rate will be 500-600 mL/min, depending on treatment modality. The electrolyte composition of the dialysis fluid is: Na 133-145 mmol/L (depending on study treatment); K 2.0-3.0 mmol/L; HCO3 25-40 mmol/L; Ca 1.25-1.50 mmol/L; Mg 0.5 mmol/L; Cl 108.0-109.5 mmol/L; glucose 5.6 mmol/L; acetate 3 mmol/L.

Dialysis modality: all treatments will be either high-flux hemodialysis, high-flux hemodialysis preceded by half an hour of isolated ultrafiltration or online postdilution high-volume hemodiafiltration. All modalities are regular and EMA approved dialysis treatments.

^{23}Na MRI: see chapter 6.

5.2 Use of co-intervention (if applicable)

Participants will use their regular co-medication, and changes in prescribed medicine by the treating physician are allowed as determined by routine patient care. Routine patient care is performed according to national (https://www.nefro.nl/richtlijnen) and international quality of care guidelines. All patients will be administered their usual dose of low molecular weight heparin anticoagulation (i.e. nadroparin or dalteparin).

5.3 Escape medication (if applicable)

Not applicable

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

The Philips Achieva 7-Tesla MRI scanner is a non-CE-marked product. Sodium-23 MR imaging with the 7-Tesla MR system will be made possible using a double-tuned radiofrequency (RF) coil consisting of a sodium birdcage surrounded by four microstrip proton elements. This non-CE marked product, manufactured by Philips Healthcare (Best, The Netherlands), is the original copy from Leiden University Medical Center (LUMC), previously utilized for research purposes (52).

6.2 Summary of findings from non-clinical studies

For more detailed information see the investigational medical device dossier (IMDD) regarding the Philips Achieva 7-Tesla MR system.

6.3 Summary of findings from clinical studies

Sodium-23 MRI is an imaging modality that offers the possibility to non-invasively quantify sodium content in tissue. The last two decades several clinical studies have applied sodium imaging to different parts of the body, including (articular) cartilage (53-59), spine (60, 61), tendon (62), muscle (63-67), heart (68-72), kidney (73-75), breast (76), and brain (77-89), as well as in several pathological conditions, including kidney disease (73-75), hyper-aldosteronism (68, 90), essential hypertension (18), diabetes mellitus (64), myocardial infarction (70-72), tumours (77, 86, 91), neurological disorders (79, 81-83, 85, 87-89) and degenerative diseases (84). More importantly, sodium-23 MRI has been previously used to investigate the removal of sodium from the skin and muscle during HD. Dahlmann et al. measured the skin and muscle concentration at the calf before and after HD and showed that TSC decreased significantly (33). The review of Zaric et al. (76) provides an overview of the current literature, including its application in (pre)clinical investigations and methodological improvements. Sodium-23 MRI imaging provides valuable information about altered metabolism or tissue function associated with pathogenesis and disease progression (76).

6.4 Summary of known and potential risks and benefits

The imposed risks of the 7-Tesla MRI scanner on the subjects are negligible. Many studies have investigated the safety of ultra-high field MRI, such as the 7-Tesla MRI (92). Moreover, in other countries these ultra-high field MRI’s have been used since the 90s. Thus far, no negative effects have been found and the only reported complaints are temporary vertigo during scanning. Exposure to a magnetic field can cause vertigo by stimulation of the

vestibular system. Furthermore, rapidly changing magnetic fields of gradient coils can cause stimulation of peripheral nerves, which can be perceived as a mild tingling or tapping sensation. Both side effects are harmless.

6.5 Description and justification of route of administration and dosage

Not applicable

6.6 Dosages, dosage modifications and method of administration

Not applicable

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Dialog iQ or Dialog+ dialysis machine

All dialysis treatments will be performed with the Dialog iQ® or Dialog+ dialysis machines (B.Braun Avitum AG, Melsungen, Germany), which are regularly used in clinical practice.

Xevonta 18/23 dialyzer

High-flux dialyzers which are suited for HDF and regularly used in clinical practice will be applied in all treatment modalities: Xevonta 23 dialyzers (membrane material: Amembris, i.e. polysulfon-based membrane with polyvinylpyrrolidone; UF coefficient: 124 ml/min/mmHg; surface area 2.3 m^2; sterilization by gamma radiation; capillary internal diameter 195 µm; B.Braun Avitum, Melsungen, Germany). In exceptional cases, the attending nephrologist can decide to treat the patient with a Xevonta 18 dialyzer (differing from Xevonta 23 only by lower membrane surface area: 1.8 m^2 and UF coefficient 99 ml/min/mmHg). In case of a polysulfon allergy, another dialyzer will be used.

Hemodiafiltration

HDF will be performed in the post-dilution mode with a target convection volume (substitution volume + net UF volume) ≥23L/session (hvHDF). Extracorporeal blood flow rate will be targeted at 350-400 ml/min, and filtration fraction (blood flow rate/convection flow rate) at 25-32%. The substitution fluid is prepared from the dialysis fluid (see below) by one additional step of filtration, before it is infused post-dialyzer into the blood. The electrolyte composition of the substitution fluid and the dialysate is identical.

Laser speckle contrast analysis: LASCA

Microvascular flow will be measured with PeriCam, in combination with equipment for local application of vasoactive stimuli (PeriLont). The PeriCam PSI System is a blood perfusion imager based on the laser speckle contrast analysis technology, also known as laser speckle contrast imaging (LSCI), and enables quantification of microvascular blood flow (arbitrary units (AU)), in this case, in the cutaneous microvascular bed of the forearm. PeriCam PSI System combines dynamic response and high spatial resolution in one instrument, providing both real-time graphs and video recordings of the tissue being studied. The integrated laser is safe to use without eye protection.

The PeriLont Micropharmacology System enables the transport of charged molecules or drugs across a tissue barrier using a non-noxious direct electrical current, also known as iontophoresis, and will be used to locally apply vasoactive stimuli to evaluate blood flow responses. The main iontophoresis applications in vasoreactivity testing involve acetylcholine and sodium nitroprusside, both widely used to assess microvascular endothelium-dependent and independent vasodilation, respectively. Each iontophoresis test requires one drug delivery electrode (LI 611 Drug Delivery Electrode) together with one counter-electrode (PF 384 Dispersive Electrode). PeriCam and PeriLont are approved medical devices with CE-marking (Certification numbers: 4131087 and 41310877-01), which will be used in accordance with attended use. Devices will be regularly maintained by the medical technology department.
The microvascular perfusion will be measured before dialysis (and after the MRI-scan). As mentioned before, in 5 of the 22 included patients MC measurements will be performed both before and after dialysis. Measurements are started after a 20-minute period of rest and acclimatization in a temperature-controlled room to avoid confounding of temperature.

PeriCam PSI Specifications: Image size (maximum): Normal Resolution model: ~ 24 x 24 cm (at 40 cm); Image acquisition rate: Up to 96/113 images per second depending on frequency of background light; Automatic frequency detection and synchronization; Image resolution: Maximum 1386 x 1036 measurement points; Normal Resolution model: 100 µm/pixel (at 10 cm); Working distance: Normal Resolution model: 10-40 cm; Dimensions and weight: Scanner head: 22 x 15 x 20 cm, ~2.4 kg; Laser safety: Class 1 per IEC 60825-1:2007 - Safe to use without eye protection; Complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant; to Laser Notice No. 50, dated June 24, 2007

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Sodium MRI see chapter 5.

Laser speckle contrast imaging provides a non-invasive approach to measure microvascular skin perfusion (93, 94). A great advantage is that the reproducibility of LASCA is superior to earlier techniques, such as laser Doppler flowmetry and Laser Doppler imaging (95, 96). LASCA has already proved to be effective in the evaluation of microvascular reactivity in several patient populations, including cardiovascular disease (94, 97-102), diabetes mellitus (103, 104), systemic lupus erythematosus (105), systemic sclerosis (106), rheumatoid

arthritis (107), peripheral arterial disease (108) and hematologic stem cell transplantation (109). Alexandrou et al. (2020) examined skin microcirculation using LASCA in end-stage renal disease patients treated with HD or peritoneal dialysis (PD). This study showed that the skin vasodilatory response was similar between PD and HD patients, but significantly impaired compared with healthy controls (110).

7.4 Summary of known and potential risks and benefits

Since LASCA is a non-invasive tool, no risks are to be expected. Iontophoresis of vasoactive substances is a safe procedure. It does not cause any skin damage and the used dosage of the vasoactive agents is too small to induce systemic drug effects (94, 111, 112). Side-effects are limited to transient erythema due to vasodilatation. The most commonly used vasoactive agents are acetylcholine (ACh) and sodium nitroprusside (SNP) (113, 114), which did not induce adverse effects in >1000 study participants (94, 97-100, 102-105, 115, 116).

Changes in the cutaneous MC induced by vasoactive agents, delivered through iontophoresis, provide valuable information about vasoreactivity and the underlying vascular physiology. Ach causes vasodilation mainly through release of nitric oxide from the endothelium and is, therefore, used to investigate endothelial dysfunction. SNP is considered a measure of non-endothelial dependent vasodilation. Using both vasoactive substances enables differentiation between different underlying pathophysiological mechanisms (114).

7.5 Description and justification of route of administration and dosage

LASCA: Application of a vasoactive stimulants Ach and SNP via a small patch on the skin is the standard route of administration using iontophoresis via the PeriLont device.

7.6 Dosages, dosage modifications and method of administration

LASCA: laser speckle assessment of microcirculation

During iontophoresis, 0.4ml of Ach (Miochol-E) 1% and 0.4 ml of SNP 1 mg/ml will be locally applied to the skin of the forearm via a small iontophoresis patch (PeriLont device). Sodium nitroprusside 60 mg powder for injection will be used to prepare the solution. It will be first dissolved in 3 mL of 5% glucose solution. This will produce a solution containing 53 mg of SNP in 3 mL. Then it will be further diluted by adding it to a 50 mL 5% glucose solution. The final solution will have a SNP concentration of 1 mg/mL.

Hemodialysis and hemodiafiltration

HD and HDF will be performed according to clinical practice. The modality order will be decided by randomization. Treatment times will be kept constant during the study. UF volume/rate will be decided on an individual basis according to clinical practice. During dialysis, sodium concentration in the dialysis fluid can be modified by altering conductivity of the dialysis fluid. Dialysate is reconstituted by dialysis machines from concentrated salts and purified water and done so through the use of conductivity cells in the dialysate flow path. These cells measure conductivity as a surrogate for ionic activity, and initiate actions within the machine (changes to proportioning, alarms and bypass). On most, if not all, modern machines, cells exist on acid and/or bicarbonate dialysate component flow paths individually, and on final dialysate flow paths pre and post the dialyser. Proprietary equations are used to determine the relative concentrations of different ions at each level of testing, with final dialysate composition computed to account for different contributions of anions and cations to the overall conductivity (117, 118).

7.7 Preparation and labelling of Non-Investigational Medicinal Product

LASCA: The iontophoresis patch will be provided by the (trial) pharmacy. The pharmacy will also be responsible for storage, labelling and distribution of the non-investigational medicinal product, in accordance with relevant guidelines. Preparation and local application will be performed by a trained physician according to standard operating procedures.

7.8 Drug accountability

LASCA: All study medications (Ach (Miochol-E) 1% and SNP 60 mg powder for injection) will be stored at the Clinical Pharmacy (AMC). Study medication will be collected from the Pharmacy by the principal investigators, research physician or research nurse. Non-used medication will be returned to the Pharmacy, where it will be destroyed following local procedures. Miochol-E will be imported through the Pharmacy purchasing department.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary end-point of the study is the amount of SSC in muscle (mmol/L).

- The SSC will be assessed with a ^{23}Na-MRI coil. The coil will be used in combination with a 7-Tesla MRI, located in the Spinoza centre at Amsterdam UMC, location AMC.

8.1.2 Secondary study parameters/end-points

- Skin SSC (mmol/L) will be measured with the ^{23}Na-MRI coil combined with the 7-Tesla MRI.

- Skin MC (expressed in arbitrary units) will be measured with a LASCA perfusion imager, which is based on the principle that the backscattered light from a tissue that is illuminated with coherent laser light forms a random interference pattern at the detector (the ‘speckle pattern’). Movement of particles inside the tissue causes fluctuations in this pattern, resulting in blurring of speckle images which are related to blood flow as the fluctuations are caused by movement of red blood cells. Skin perfusion and vasoreactivity will be measured.

- Hemodynamics: Intradialytic BP will be assessed by automatic arm-cuff measurements attached to the dialysis machine. BP will be measured 4x/hour during all treatments. Additionally, interdialytic BP will be evaluated using an automatic BP-monitor, which is provided as a gift upon inclusion. Patient will be asked to measure BP at home 3x/day during one interdialytic day between the second and third dialysis treatment in the last week of each treatment period.

- Patient experience: patient reported outcome measures (PROMs) as indicated by the Dialysis Symptom Index (DSI) will be documented. Furthermore, as sodium may influence thirst, interdialytic thirst will be estimated using the Thirst Distress Scale.

Lastly, patients will be asked to rate their quality of life on a visual analogue scale (EQ-5D-5L VAS). All PROMs will be collected at the end of each treatment period.

- Markers of cardiac damage: at t0 and t240, CK-MB will be measured in blood drawn from the arterial line of the extracorporeal circuit (ECC)

- Markers of inflammation: at t0 and t240, high-sensitive C-reactive protein (hsCRP), interleukin-6 receptor (IL-6R), soluble CD163, and soluble intercellular adhesion molecule-1 (sICAM-1) will be assessed using blood from the arterial line of the ECC.

- Markers related to sequestered sodium content: at t0 and t240, serum glycosaminoglycans (GAGs), syndecan-1 and vascular endothelial growth factor C (VEGF-C) will be assessed using blood from the arterial line of the ECC.

- Depending on a pilot study, extracellular vesicles (EVs) as markers for organ damage will be assessed using blood from the arterial line of the ECC.

8.1.3 Other study parameters

- At baseline, demographic data, medical history, stimulant use (i.e. tobacco, alcohol, drugs), medication and dialysis-related parameters will be assessed. Regular laboratory values will be extracted from the electronic patient file.
- Hematocrit levels will be measured before and after dialysis during the last dialysis session of each modality to adjust for hemoconcentration.
- Hemoglobin, platelet and white blood cell count will be measured before and after the last dialysis session of each modality
- Before the first treatment of each modality, serum sodium will be assessed; and averaged with 2 other monthly pre-dialysis values. These measurements coincide with the scheduled blood draws at baseline and at the end of each treatment period.
- At baseline, diet will be assessed with the Dutch Food Frequency Questionnaire (FFQ-tool) and with ‘De Zoutmeter’ of the Nierstichting (estimating salt intake).

8.2 Randomisation, blinding and treatment allocation

In the present cross-over study, all patients will be subjected to the 5 described treatment modalities in a random fashion. Randomisation will be performed by computer software (Castor). Due to the differences in setup between HD and HDF, such as an additional set of lined and a second pump in HDF, and the fact that the treatment modality is displayed on the dialysis machine screen, it not possible to blind patients or the researcher. However, the data

analyst (CRZ) will be blinded for the treatment arm by encrypting the data after extraction from Castor.

8.3 Study procedures

Patients will be dialysed according to their routine schedule (3 times a week), with different treatment modalities in random order (figure 1). At baseline, laboratory parameters and the salt intake will be evaluated. At the end of each treatment period, skin MC, the sequestered salt content, laboratory parameters and PROMs will be assessed. BP will be measured during all treatments.

8.3.1. Assessment of microcirculation

Skin MC will be measured with a laser speckle contrast analysis perfusion imager. Application of vasoactive stimulants via a small patch on the skin is standard route of administration using iontophoresis via the PeriLont device. During iontophoresis 0.4ml of Ach (Miochol-E) 1% and 0.4 ml of SNP 1mg/ml will be locally applied to the skin via a small patch.

8.3.2 Assessment of the sequestered sodium contact

The SSC will be assessed with a ^{23}Na-MRI coil. The coil will be used in combination with a 7-Tesla MRI, located in the Spinoza Centre at AUMC. The sodium coil (double tuned RF coil) will be put on the lower leg of a patient. The procedure will take approximately 1 hour.

8.3.3 Assessment of laboratory parameters

Laboratory tests will be performed on blood samples drawn from the arterial line of the ECC (no venipuncture). At week 2 of each modality, plasma sodium will be determined at the start of dialysis. In total, 181 mL will be extracted during the 20 weeks of the study. Given the low volume of blood over a prolonged period of time under regular follow-up of the Hb (usually every month) with, if necessary, an increase in prescribed dose of recombinant erythropoietin, a decrease in hemoglobin levels due to the study is not to be expected.

8.3.4 Assessment of hemodynamic parameters

BP will be assessed before dialysis, then every 15 minutes and after dialysis (i.e. 17 measurements in 4 hours) with a manometric cuff in different sizes dependent on patients' body size. The cuff is connected to the dialysis machine (automatic BP measurement cuff, B. Braun Avitum AG, Melsungen, Germany). In addition, patients will be asked to measure BP at home 3 times a day between the second and third dialysis treatment in the last week of each modality. Patients will receive a calibrated and clinically validated device, Omron M6

Comfort (product number: HEM-7321-E, Omron Healthcare Europe BV, Hoofddorp, The Netherlands), as a gift upon inclusion to measure BP at home. This device offers the capability to perform three consecutive measurements at 30 seconds intervals and automatically calculate the mean with a single press of the start button. Additionally, it has a memory capacity of 200 readings.

8.3.5 Assessment of patient reported outcomes

Patients will be asked to answer three questionnaires at the end of each treatment period. The following questionnaires will be used: the Thirst Distress Scale, the DSI and the EQ visual analogue scale. See Appendix.

8.3.6 Assessment of other parameters

At the end of each treatment mode, tympanic body temperature will be assessed before and after dialysis (10x during the study).

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time without reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. There are no specific criteria for withdrawal.

8.5 Replacement of individual subjects after withdrawal

Not applicable, as the number of patients to include accounts for a dropout of 25%.

8.6 Follow-up of subjects withdrawn from treatment

Withdrawn patients will be followed by their own nephrologist (who sees the patient every week according to the current Dutch guideline (https://www.nefrovisie.nl/wp-content/uploads/2020/01/Normendocument-Visitatie-Dialysecentra-5.1.pdf).

8.7 Premature termination of the study

If serum sodium changes to ≥148 mmol/L or ≤130 mmol/L, participants will be withdrawn.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

As none of the study treatments is experimental, no specific adverse events related to the current study are to be expected. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the interventions. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any undesirable medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

Elective hospital admission will not be considered as a serious adverse event.
The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial

preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable (this is not a study with an investigational medicinal product).

9.3 Annual safety report

Not applicable (this is not a study with an investigational medicinal product).

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs will be reported till end of study.

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not applicable (standard treatments are to be compared; no survival end point).

10. STATISTICAL ANALYSIS

Descriptive data will be shown quantitatively as mean (standard deviation), median (interquartile range) or number (percentage), as dictated by data type. Missing data will not be replaced due to the low number of subjects.

10.1 Primary study parameter(s)

The SSC in muscle generated by the MRI is given in mmol/L (a continuous variable). First, we will assess whether this data is normally distributed using descriptive statistics and graphs. If the data is normally distributed, the difference in muscle SSC between HDF with a zero sodium balance (DNa = PNa) and HD with a zero sodium balance (DNa = PNa) will be assessed using a matched paired t-test. If the data is not normally distributed, the non-parametric Wilcoxon signed-rank test will be used. Differences in SSC between the 5 dialysis modes will be analyzed using LMMs. For generalized linear mixed models (LMMs), a random slope, random intercept or both will be used, depending on the lowest Aikaike's Information Criterion.

10.2 Secondary study parameter(s)

Most secondary end-points are continuous variables (skin MC, skin SSC, BP, markers of cardiac damage, endothelial damage, inflammation and markers related to the sodium balance and SSC). These will be analysed using ANOVA, Friedman tests and/or generalized LMMs, depending on data distribution. Changes between pre- and post-dialytic values will be evaluated using similar tests. For LMMs, a random slope, random intercept or both will be used, depending on the lowest Aikaike's Information Criterion.
Descriptive data will be used to assess potential differences in patient-reported outcomes.

10.3 Other study parameters

Not applicable

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the WMA Declaration of Helsinki (October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The protocol will be judged and approved by the Medisch Ethische Toetsingscommissie (medical ethical committee) of the Amsterdam UMC (https://www.vumc.nl/afdelingen/METc/). Any protocol amendments will be approved by the METc and communicated to the trial participants, the trial registry, the financial contributors and the investigators. This study will be conducted according to good clinical practice (GCP) guidelines. The research doctors shall inform patients about the explanatory document and the consent forms are signed and dated by the subjects individually.

11.2 Recruitment and consent

Patients will be informed about the study by the attending nephrologist. If a patient expresses interest in participating, one of the researchers (the study physician, the research nurse or the primary investigator) will provide them with more detailed information, including a Patient Information File (PIF). The PIF will be reviewed by the Nierpatiëntenvereniging (Society of Kidney Patients) and approved by the METc (PIF and informed consent forms: see appendix). After a minimum period of 1 week, written informed consent will be obtained from the participant by one of the researchers (the study physician, the research nurse or the primary investigator), before their inclusion in the study. Participants can withdraw from the study at all times without giving reasons. Such a decision will not interfere with their medical care. In the case of treatment-related problems, patients will be withdrawn from the study. Both HD and HDF are regular and EMA-approved treatment modalities for ESKD patients.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

Several efforts will be asked from the study participants. First, a ^{23}Na MRI measurement will be performed before the last treatment of each study arm (5 in total). Five out of the 23 participants will be asked to undergo an additional MRI after the last treatment of each study arm. As this technique is only available at AUMC, patients will be dialyzed at their own dialysis facility (Niercentrum aan de Amstel or Dianet (location Amsterdam)) and transport to the Spinoza Centre (Amsterdam UMC, location AMC) will be arranged by the study team and

paid from the study budget. LASCA will be measured before the last session of each modality (5 in total). Again, 5 patients will be asked to measure their MC both before and after dialysis. At the last treatment session of every modality, other measurements will be performed as well. Then, to assess their well-being, participants will be asked to fill in three surveys in the last week of each treatment arm. Diet, specifically salt intake, will be evaluated at baseline. At the final treatment of each modality, blood will be drawn before and after treatment. To minimize inconvenience, the research physician or research nurse will guide them through the last day of each treatment period. No adverse effects of the study are to be expected. However, the burden of assessments is considerable. Therefore, the protocol has been reviewed by patient referees during the grant application and adapted according to their advice.

Altogether, we feel the potential results are worth the efforts. With the results of this study, we gain more insight in the effect of various dialysis sodium contents and the dialysis modality used on the microcirculation, sequestered sodium content, markers of organ damage, blood pressure, patient well-being during dialysis and thirst. Using this information, we may be able to optimize and individualize dialysis prescription (modality and/or dialysate sodium content) to ultimately improve both quantity and quality of life of these patients.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO (Centramed, policy number 626.107.174). The sponsor (also) has an WMO subject insurance (Centramed, policy number 624.100.044), which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within the following 4 years.

11.6 Incentives (if applicable)

Participants will receive compensation for the study-related time investment based on the minimum wage (13.86 euros/hour). This compensation covers the additional time required for study-related procedures (including travel time), but does not cover time spent on dialysis treatments, which is part of standard care. Each measurement day requires up to 2.5 hours, consisting of 1 hour for an MRI-scan, up to 1 hour for LASCA measurements, and 30 minutes for travel. The compensation per measurement day is rounded to 40 euros. For 17 participants, this amounts to a total of 200 euros for 5 measurement days. For 5 participants who undergo MRI scans and LASCA measurements both before and after dialysis, the time

investments is doubled to 5 hours, resulting in 80 euros/measurement day. Consequently, these participants will receive 400 euros for 5 measurements days.
Additionally, taxi transportation to the Spinoza center will be reimbursed and arranged on the 5 assessment days. Furthermore, patients will also receive an automatic blood pressure monitor (Omron M6 Comfort, HEM-7321-E) as a gift to facilitate blood pressure measurements at home.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be registered by a dialysis nurse or a research physician in an electronic CRF in Castor EDC according to the GCP guidelines (GCP section 4.9). The following data will be collected: demographic information (including ethnicity), anthropometric data (such as weight, length and BMI), medical history, medication use, laboratory results, parameters related to dialysis treatment, data from physical assessments (including blood pressure and heart rate), outcome data (SSC and cutaneous microcirculatory perfusion findings).
Information about ethnicity will be gathered through interview in order to provide a detailed description of our study population. This is essential for determining generalizability of the results, since different ethnic groups can exhibit variations in health outcomes and treatment responses.
Pseudonymisation will be applied to all personal data and biospecimens. Each participant will receive an exclusive subject identification code (e.g. ID1, ID2 etc.) according to their inclusion order. Access to the subject identification list will be limited to the principal investigator, research physician and research nurse.
The storage duration for the collected biospecimens will be 5 years; the storage duration for personal data will be 15 years. The handling of personal data complies with the EU General Data Protection Regulation and the Dutch AVG (Act for Implementation of the General Data Protection Regulation). To prevent data breach several measures will be applied, including pseudonymisation, data minimization, purpose limitation (i.e. data that is not considered to be compatible with the study purpose will not be collected) and protection against unauthorised or unlawful processing and against accidental loss, destruction or damage. This will be achieved by limited access, secure storage of personal data (Castor EDC) and requirement of informed consent. If a data breach occurs, it will be promptly reported to the Data Protection Officer of the Amsterdam UMC, who will then notify the Dutch Data Protection Authority (Autoriteit Persoonsgegevens) within 72 hours.

12.2 Monitoring and Quality Assurance

Monitoring will be performed by the Clinical Monitoring Center of Amsterdam UMC. During the selection of the participants, the Informed Consent forms will be checked. Furthermore, during the onsite monitoring, Source Data verification will be performed (check if the data in the Case Report Forms (study forms and questionnaires) are consistent with the source data (patient files, lab results, etc). As the risk of the study is low (as compared to the risk of the

routine treatment), this verification will be performed in 5% of the treatments. Data that will always be checked are in- and exclusion criteria and the primary outcomes of the study.

12.3 Amendments

Amendments are changes made to the research after the original research dossier has been approved by the accredited METC. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the investigators. All other amendments will be notified to the METC and only implemented after approval. Substantial amendments will also be notified to the competent authority.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within 8 weeks. The end of the study is defined as the last patient's last visit. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results, including any publications/abstracts, to the accredited METC.

12.6 Public disclosure and publication policy

The trial will be registered in a public trial registry before the first patient is recruited. All results will be published in international, peer-reviewed journals. Financial contributors will be informed about the study design and interpretation of the data. They will not have influence on the analysis of the data and/or publication of the results, irrespective or their nature.
In accordance with Article 4 of the CCMO Guideline for Assessing Research Contracts, we are committed to publishing the results of this study, irrespective of the outcome, within twelve months after completion of the study.

13. STRUCTURED RISK ANALYSIS

In general: as HD or HDF is a life-saving treatment for patients with ESKD, and several aspects of dialysis prescription have not been studied prospectively, or the effects of dialysis prescription on clinical end points is not thoroughly known, it is necessary to perform clinical studies (instead of animal or ex-vivo studies). Furthermore, it is virtually impossible to simulate clinical dialysis in real life patients with multiple comorbidities, primary kidney disease and ages.

13.1 Potential issues of concern

Sodium concentration in dialysis fluid: HD or HDF with sodium in dialysis fluid (DNa) = serum sodium (PNa) or DNa is 3 mmol/L lower than PNa.
In clinical practice, either standard DNa is prescribed, varying between dialysis facilities (<138 to >142 mmol/L), or individualized DNa. It is unknown which policy is related to better outcome (119). A systematic review showed no evidence of superiority of higher or lower DNa (120). In a prospective study, DNa 135 mmol/L was compared with 140 mmol/L (regardless of PNa), and no differences in LV mass were observed after 12 months (121). In both the systematic review and the RCT, interdialytic weight gain was somewhat higher in patients treated with higher DNa, and intradialytic hypotensive episodes somewhat more prevalent in patients treated with lower DNa.

1. Identify hazards:
   Increase in incidence of intradialytic hypotension with DNa –3 mmol/L as compared to PNa.
2. Assess risks:
   All participants might be harmed by the occurrence of IDH, regardless of the DNa; the risk is probably slightly higher if DNa –3 mmol/L as compared to a DNa equal to PNa. However, it must be acknowledged that both lower DNa (-3mmol/L) and a DNa equal to PNa are currently applied in clinical practice and that it remains unclear which approach is more beneficial. Furthermore, IDH is the most prevalent intradialytic complication, irrespective of the DNa used.
3. Evaluate risks and take precautions:
   As IDH is one of the most frequent complications of dialysis, all nurses and physicians have been trained to recognize and treat such episodes. If an IDH episode occurs, nurses will stop or reduce UF and/or administer intravenous saline until BP normalizes. If IDH episodes occur too often, the patient can be withdrawn from the study.

4. Record findings:
   IDH episodes are a secondary endpoint of the study and as such will be recorded.
5. Review assessments:
   The number of IDH-episodes/treatment is a secondary end point of the study, and will be reported.

Na^{23} MRI: several studies have been performed in different patient populations evaluating SSC. No adverse events of the MRI procedure itself were described (33). Naturally, MRI procedures might be harmful for people with an ICD, cardiac pacemaker, metallic foreign body in the eye or an intracerebral aneurysm clip. Furthermore, claustrophobic patients might experience discomfort and fear in the MRI. Those patients will be excluded.

1. Identify hazards
   MRI procedures might be harmful for people with an ICD, cardiac pacemaker, metallic foreign body in the eye or an intracerebral aneurysm clip. Furthermore, patients with claustrophobia might experience discomfort and fear in the MRI.
2. Assess risks
   Pacemakers might be deregulated resulting in cardiac arrhythmia, metallic foreign bodies might change position resulting in local harm; patients might experience fear during the procedure.
   There are no risks specific for Na-MRI (as compared to MRI in general).
3. Control risks
   Patients with a contra-indication to MRI will not be included in the study.
4. Record findings
   Patients will be accompanied by the research physician or research nurse during the MRI. Any unexpected finding during or after MRI will be recorded.
5. Review assessments
   Not applicable.

13.2 Synthesis

Hemodialysis and hemodiafiltration are life-saving treatments. The medical devices involved (dialysis machines, ECCs, needles, acid and bicarbonate concentrates for the online production of dialysis fluids) all do have a CE mark and are not the subject of study. Hence, a structured risk analysis for HD and HDF is not wanted. The possible risks of varying sodium content of dialysate are encountered on a day-to-day base in clinical practice. As no clear evidence on the best sodium content is available, and dialysate sodium will only be 0-3

mmol/L different from the plasma sodium, this study will add to guiding clinicians in decision making in this respect.

LASCA: many studies with LASCA have been performed in different patient groups, see chapter 7.4. As this in a non-invasive, CE marked medical device for diagnostic purposes, no formal risk assessment is needed.

In summary: the present study investigates variations in HD/HDF treatment (different sodium content of the dialysis fluid, isolated UF), that are in a non-systematic way already part of clinical practice. The main risks (IDH, increase in thirst, increase in interdialytic weight gain) are encountered in daily clinical practice on each dialysis ward. Furthermore, they will be recorded during the study.
The amount of blood drawn will be limited (181 ml during 20 weeks), and management of renal anemia is part of clinical care for any dialysis patient, who is seen on a weekly base by the attending nephrologist (if Hb levels drop, dosage of erythropoietin receptor stimulating agents will be increased, or iv iron will be administered, both during treatment and in the ECC). MC and SSC will be assessed in a non-invasive manner. Patients with a contra-indication (this will be checked meticulously) to MRI will not be included in the study.
Hence, the risks seem minimal, and if any unusual effects occur, these most probably will be noticed by the nurses (contact: 3 times/week) or physicians (contact: once a week) at the dialysis ward.

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