Randomized controlled trial of individualized dialysate cooling for cardiac protection in hemodialysis patients

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Key Words: aortic distensibility, cardiac imaging, cardiovascular disease, dialysate cooling, hemodialysis, randomized clinical trial,

Running head: Randomized clinical trial of dialysate cooling

Publisher version available: http://cjASN.asnjournals.org/content/early/2015/05/10/CJN.00200115.abstract
Abstract

Background and objectives
Cardiovascular disease is the most common cause of death in hemodialysis (HD) patients. HD associated cardiomyopathy is appreciated to be driven by exposure to recurrent and cumulative ischemic insults resulting from hemodynamic instability of conventionally performed intermittent HD treatment itself. Cooled dialysate reduces HD induced recurrent ischemic injury but whether this confers long-term protection of the heart in terms of cardiac structure and function is not known.

Design, setting, participants, and measurements
Between September 2009 and January 2013, 73 incident HD patients were randomly assigned to a dialysate temperature of 37°C (control) or individualized cooling at 0.5°C below body temperature (intervention) for 12 months. Cardiac structure, function and aortic distensibility were assessed by cardiac magnetic resonance imaging. The mean between-group difference in delivered dialysate temperature was 1.2±0.3°C. Treatment effects were determined by the interaction of treatment group with time in linear mixed models.

Results
There was no between-group difference in the primary outcome of left ventricular ejection fraction (1.5% [95% confidence interval -4.3% to 7.3%]). However, left ventricular function assessed by peak-systolic strain was preserved by the intervention (-3.3%[-6.5% to -0.2%]) as was diastolic function (measured as peak diastolic strain rate, 0.18s⁻¹ [0.02s⁻¹ to 0.34s⁻¹]). Reduction of LV dilation was demonstrated by significant reduction in left ventricular end-diastolic volume (-23.8ml [-44.7ml to -2.9ml]). The intervention was associated with reduced left ventricular mass (-15.6g [-29.4g to -1.9g]). Aortic distensibility was preserved in the intervention group (1.8mmHg⁻¹×10⁻³ [0.1mmHg⁻¹×10⁻³ to 3.6 mmHg⁻¹×10⁻³]). There were no intervention-related withdrawals or adverse events.

Conclusions
In patients new to HD, individualized cooled dialysate did not alter the primary outcome but was well tolerated and slowed the progression of HD associated cardiomyopathy. As cooler dialysate is universally applicable at no cost, the intervention warrants wider adoption or confirmation of these findings in a larger trial.
Introduction

Over two million people worldwide require on-going dialysis to sustain life. Most patients endure unpleasant dialysis-related symptoms and generally have a poor quality of life. Dialysis patients continue to have a lower life expectancy than many cancer patients, with cardiovascular disease the leading cause of death. Several cardiovascular disease therapies effective in the general population have been entirely ineffective in hemodialysis (HD) patients. However, cardiovascular disease in dialysis patients is not solely caused by traditional risk factors or the uremic milieu (1). HD itself causes recurrent and cumulative ischemic injury to the heart, brain and other organs (2). Recurrent HD induced myocardial stunning results in long-term contractile dysfunction, abnormal ventricular morphology and increased mortality (3-5).

The reduction in intradialytic hypotension by cooling the dialysate was first demonstrated by the original pioneering work of Maggiore (6). However, this therapy remains greatly underused. In the first half of 2014, 24% of in-center HD treatments had dialysate temperature of <36.5°C in a sample of a large dialysis organization in the United States (Len Usvyat, Fresenius Medical Care, personal communication). We have demonstrated in several small short-term pilot studies that improvement in hemodynamic tolerability of HD is associated with marked reduction in myocardial stunning (7-9). The use of extremely low temperature dialysate (34-35°C) is effective but limited by cold symptomatic intolerance. The cardio-protective effect and cold symptom tolerability can be optimized by an individualized approach to dialysate temperature prescription (10).

We recently reported that an individualized dialysate cooling strategy is effective in reducing HD associated progressive white matter brain injury (11). This trial aimed to test whether dialysate cooling could provide long term cardiac protection and abrogate progressive morphological and functional changes characteristic of HD associated cardiomyopathy in patients new to conventional thrice weekly HD.

Methods

Study design and treatment regimen

The trial was conducted in accordance with the Declaration of Helsinki of 1975 and as revised in 1983. The trial design was a prospectively registered (ISRCTN00206012, 15 October 2009), multicenter, randomized,
controlled, open-label, blinded endpoint (PROBE) study and the protocol has been published (12). Nottingham Ethics Committee approved the protocol and all patients gave written informed consent.

Participants

Participants were recruited from the HD centers of four university teaching hospitals in the United Kingdom. Inclusion criteria were 16 years of age or greater, being within 180 days of commencing in-center HD treatment three times per week and with capacity to consent for the trial. Exclusion criteria were inability to tolerate cardiac magnetic resonance imaging (CMR) due to claustrophobia, contraindications for CMR, pregnancy or lactating and New York Heart Association grade IV heart failure.

Randomization and blinding

Patients were randomized 1:1 by a computer-generated sequence placed into sealed envelopes by an independent statistician. As the HD monitor was iteratively programmed per treatment session with potentially visible settings, it was not pragmatic to reliably maintain blinding for 12 months. As per the PROBE design all cardiac imaging analyses were centrally conducted blinded to patient details or treatment group allocations.

Dialysis Intervention

The control group used a dialysate temperature of 37°C for 12 months. The intervention group used an individualized cooled dialysate temperature for 12 months. The latter was set at 0.5°C less than the patient’s, determined from the mean of 6 prior treatment sessions with a tympanic thermometer, between a minimum of 35°C and a maximum of 36°C. The allocated dialysate temperature remained unchanged for the study period. This ensured a minimum between-group temperature separation of 1°C in the event that a participant had a mean temperature greater than 36.5°C. Preliminary review of electronically recorded data indicated the mean allocated intervention group temperature was likely to be 1-1.5°C lower than the control group. Protocol adherence was regularly assessed and recorded by HD center staff.

Concurrent Treatments
Patients in both trial arms had standard management with dry weight prescribed by the clinical team according to local protocol including three-times weekly HD to achieve an equilibrated Kt/V urea >1.1, a standardized Kt/V urea of >2.0 and a treatment time of between 3.5 hours and 4 hours. HD treatments used low-flux polysulfone dialyzers, 1.8-2.0 m$^2$, (LOPS 18/20; Braun Medical Ltd, Sheffield, United Kingdom). Dialysate composition was sodium 136-138mmol/l; potassium 2mmol/l; calcium 1.25mmol/l; magnesium 0.5mmol/l; bicarbonate 32-38mmol/l; glucose 5.6mmol/l and acetate 3mmol/l. Dialysate flow rate was 500 mL/min and blood pump speed 250-450 mL/min using anticoagulation by unfractionated heparin.

Data collection

Imaging studies by CMR occurred on a midweek post-dialysis day at baseline and 12 months, to avoid the long inter-dialytic break after the weekend, when patients are most hypervolemic (13, 14). Studies were conducted at two centers using 1.5 Tesla scanner (GE Signa HDxt, GE Healthcare, Milwaukee, United States) by validated methods (15). Acquisition parameters achieved a spatial resolution of <2mm and a temporal resolution of 20-40ms. Radiofrequency tagging used a spatial modulation of magnetization sequence applied at basal, mid-ventricular and apical levels. An oblique sagittal orientation was acquired to image the aorta in long axis and a 5 mm thick axial cine was acquired at the level of the pulmonary artery bifurcation.

Body composition

Volume status was assessed by segmental multiple frequency bioelectrical impedance measurements at baseline and 12 months, using tetrapolar 8-point tactile electrodes (InBody S20, Biospace, Seoul, South Korea) (16). Hypervolemia was estimated by the ratio of extracellular water to total body water (17).

Data Analysis

Planimetry, as previously described, was used to determine LV mass, volumes and EF with cvi42 software, version 4.0.2 (Circle Cardiovascular Imaging, Calgary, Canada) (15). Values were indexed to body surface area using the method described by Mosteller (18).
LV Strain Analysis

Direct assessment of regional motion within myocardial tissue by LV strain has inherently greater sensitivity and specificity to detect LV dysfunction than EF, which infers function from the bulk effect of LV boundaries. Greater LV strain denotes better LV function. Reduced LV strain precedes LV dilation, LV hypertrophy and reduced EF in a range of cardiomyopathies (19). Tagged CMR is a highly reproducible, reference standard technique to quantify regional LV strain (19). This involves changing magnetization during CMR image acquisition to ‘tag’ the LV with a grid pattern. Software then tracks the motion of these tags to quantify the magnitude (strain) and velocity (strain rate) of tissue motion. Mid-wall circumferential strain was measured in tagged LV short-axis images using validated software (HARP 3.0, Diagnosoft, Palo Alto, California, USA) as depicted in Figure 1A-1D (20). Values were mapped to the 16 segment model of the LV recommended by the American Heart Association (21) then averaged to derive global peak systolic-strain (%), peak-systolic strain-rate (s⁻¹) and peak diastolic strain rate(s⁻¹).

Aortic distensibility

Aortic distensibility analysis is depicted in Figure 1E-1F as previously described (22). The ascending thoracic aortic area was manually traced using Jim version 6 (Xinapse software, UK) and graphically represented against time. Aortic distensibility was determined by a validated formula:

\[(\text{maximum aortic area} - \text{minimum aortic area})/(\text{minimum aortic area} \times \Delta P),\]

where \(\Delta P\) is the mean of three brachial pulse pressure readings performed during CMR (23).

Statistical Analysis

Descriptive statistics for continuous variables were tested for normality and summarized using mean±SD or median (interquartile range; 75th minus 25th centile). Discrete variables were summarized by proportions. Treatment outcomes were estimated by the interaction of treatment group with time in linear mixed-effects models. Patients were specified as random intercepts adjusted for study center, age, sex and diabetes status and the covariance matrix was unstructured. Treatment effect estimates for segmental strain also accounted for correlations of LV segments within patients. As outcomes were assessed at a single follow-up time, these
models produced essentially identical results to ANCOVA. All pre-specified analyses used a two-sided significance at p<0.05 with the per comparison error rate controlled using the correction described by Sidak (24). To minimize bias in treatment outcome estimates, analyses were performed by the intention-to-treat principle with multiple imputation of missing follow-up CMR data as detailed in Supplementary Materials (25). Post-hoc explanatory analyses of interdialytic weight gain and pre-dialysis blood pressure were done without data imputation using a two-sided significance level of p<0.001. SPSS version 21.0 was used for all analyses.

**Primary Study Outcome**

The pre-specified primary outcome was the change in resting EF by CMR at 12 months compared to baseline between the intervention and control group.

**Secondary Study Outcomes**

The pre-specified secondary outcomes were LV mass and LV volumes, global peak-systolic strain, global peak-systolic strain rate, global peak-diastolic strain rate and aortic distensibility.

**Rationale for study outcomes**

No applicable strain based data in HD patients were available prior to this study. We utilized data from a previous natural history study of patients well established on HD, using the observed 13% reduction in EF by echocardiography over 12 months seen in patients experiencing HD-induced LV wall motion abnormalities vs. those who did not (3). Prior data supported EF as a predictor of cardiovascular mortality with a change of 5% approaching the minimum clinically important difference (26). CMR is the reference technique to determine EF (27). As some clinical therapy trials for heart failure reported mortality reductions with unaltered EF, we augmented assessment of EF with LV mass, LV strain and aortic distensibility, characterizing potentially subclinical cardiomyopathy. Several studies in HD patients showed an independent association of changes in LV mass with cardiovascular mortality (28, 29). Recent studies determined that systolic strain and diastolic dysfunction predicted all-cause mortality or cardiovascular events in HD patients with normal EF (30, 31). Both HD and non-HD population studies show that aortic distensibility independently predicts future cardiovascular events (22, 32).
Sample size estimation

Assuming a mean EF of 67% in the control arm and Standard Deviation of 6%, a study of 64 participants would resolve a 5% difference in EF between groups with 90% power at 5%, 2-sided significance level (15). The trial would require 52 participants to retain 80% power for a 5% difference in EF under the same assumptions. Allowing for study attrition of 10%, target recruitment was set at 72 participants.

Monitoring for Adverse Events

Treatment-related adverse events were summarized for each treatment group.

Results

Patient characteristics

Seventy-three patients were enrolled into the trial and randomized, 54 patients were analyzed (28 control, 26 intervention, age 60±24 years). The Consolidated Standards Of Reporting Flow Chart is in Figure 2. Baseline characteristics were similar between the two randomized groups (Table 1).

Adverse events and protocol adherence to treatment allocation

There were no intervention-related withdrawals in the intervention group. Two patients in the control group did not tolerate a dialysate temperature of 37°C and had dialysate temperature lowered to 36°C by the clinical team independent of the investigators. They were included in all analyses by their original treatment allocation. The mean delivered dialysate temperature in the intervention group was 35.8±0.3°C achieving a 1.2±0.3°C between-group separation.

The pre-specified treatment outcomes are summarized in Table 2 and additionally represented as standardized effect sizes in Figure 3.

Primary outcome
There was no statistically significant change in EF between control and intervention groups (1.5% [95%CI, -4.3% to 7.3%])

**Secondary outcomes**

The intervention was associated with significant reductions in LV mass (-15.6g [-29.4 to -1.9g], Table 2). The intervention was also associated with a significant reduction in LV end-diastolic volumes compared with no change in the control group (-23.8ml [-44.7ml to -2.9ml], Table 2). Global LV systolic function by peak-systolic strain was preserved in the intervention group and significantly reduced in the control group (within-group change 0±7.6% in intervention vs. 3.3±6.9% decrease in control, difference -3.3% [-6.5% to -0.2%]). Similarly, the intervention preserved peak-systolic strain rate (-0.2s⁻¹[-0.3s⁻¹ to -0.08s⁻¹]). There was also preservation of diastolic function in the intervention group relative to the control group (0.18s⁻¹ [0.02s⁻¹ to 0.34s⁻¹]). Regional LV analysis showed significant segmental strain reduction in 10 of 16 LV segments in the control group compared with no significant change in the intervention group (Figure 4). Aortic distensibility was preserved in the intervention group and significantly decreased in the control group (-0.3±2.6mmHg⁻¹×10⁻³ intervention vs. -2.3±0.6mmHg⁻¹×10⁻³ control, difference 1.8[0.1mmHg⁻¹×10⁻³ to 3.6mmHg⁻¹×10⁻³]).

**Post-hoc explanatory analyses**

Analyses of volume status by bioimpedance, interdialytic weight gain and pre-dialysis mean arterial blood pressure showed no significant differences between groups over 12 months and no differential effects of baseline LV mass on treatment effects (as detailed in Supplemental Material).

**Discussion**

This randomized controlled trial demonstrates that the use of individualized cooler dialysate results in preservation, or improvements, in important structural and functional cardiovascular abnormalities in HD patients. The intervention was operationally simple to deliver, fitting well within an existing model of care for HD patients and providing a substantial differential in delivered temperature. The existence of this differential reinforces the considerable variation in core temperature encountered within dialysis patients. Although a significant difference in the primary outcome of EF was not evident, it should be emphasized that the
intervention was associated with preservation or improvement in an otherwise complete range of pre-specified and clinically relevant secondary outcomes. This comprehensive assessment was made possible by the application of advanced CMR imaging, to directly quantify included measures of LV systolic and diastolic function, aortic distensibility and left ventricular mass. The observed effect sizes are of a magnitude previously associated with changes in cardiovascular mortality in HD patients (28, 33).

The more recent application of strain analysis has confirmed the prognostic value when EF is preserved, supporting that EF may not have been the optimal primary cardiac imaging based outcome (30, 31). In this trial there was a clear and consistent signal of both preservation of function directly measured by LV strain and inferred from reductions in LV mass and LV dilation, that the cooling intervention was effective at protecting the heart.

Left ventricular hypertrophy is an established predictor of cardiovascular mortality in HD patients and reducing LV mass is recognized as an important therapeutic goal (28). Conventional approaches to achieve this have largely relied on the control of pressure and volume overload. Daily dialysis is an effective approach in this respect, and the Frequent Hemodialysis Network trials reported HD six times weekly led to an adjusted mean reduction in LV mass (~13.8g, 95% CI -21.8g to -5.8g) and LVEDV (~11.0%, 95% CI -16% to -6%) with no change in EF over 12 months compared to conventional three-times weekly HD (34, 35). This current trial reports a similar reduction in LV mass (~15.6g, 95%CI -29.4g to -1.9g) and LVEDV (-23.8ml, 95% CI -44.7ml, -2.9ml) with no change in EF over 12 months. In keeping with the Frequent Hemodialysis Network trials, the magnitude of the LV mass reduction was partially dependent on baseline LV mass (36). It is of particular interest in the current trial that the observed reductions of LV mass cannot be explained by differences in pre-dialysis blood pressure, fluid status or inter-dialytic weight gain. However, the myocardium is capable of responding to a wide variety of other remodelling signals (37, 38). Repetitive cardiac ischemia leads to cardiac hypertrophy and fibrosis and reduced HD induced ischemia by cooler dialysate may exert a potent influence (39, 40). Directly protective benefits of lower temperatures on cardiac myocytes have also been described (41). Furthermore, there are important interactions between the LV and aortic function (so-called ventriculo-vascular coupling). Prior data showed reduced aortic distensibility or LV hypertrophy increased myocardial oxygen demand or reduced diastolic coronary artery perfusion (42). Thus, patients with lesser aortic distensibility or
increasing LV mass are more vulnerable to repetitive HD induced cardiac ischemia (43, 44). The preserved aortic distensibility over 12 months in the intervention group compared to significant reductions in the control group may have contributed to the observed cardiac effects (22, 32). The interactions between intra-dialytic events and fluid/volume status are complex and additional investigations are warranted to understand the mechanisms by which dialysate cooling might attenuate the progression of cardiomyopathy in HD patients. Future trials might be predicated on clinical outcomes and run as registry-based, cluster randomized controlled trials. Based on current event rates, 3 years follow-up and average facility size of 121 patients, we estimate a study would require 14 clusters per arm to detect a 20% reduction in a primary composite endpoint of all-cause mortality or hospitalizations with a major cardiovascular event. However, as dialysate cooling is universally applicable without cost, well tolerated, with no apparent harm and probable benefits to both the heart and the brain (11); dialysis clinicians and patients may alternatively consider that these data approach, “proof beyond a reasonable doubt” and adopt wider use without further trials.

**Limitations**

This first randomized trial of dialysate cooling has important limitations. The trial was not designed to reliably estimate ‘hard’ clinical outcomes such as hospitalization rates or mortality. Data on nursing interventions and intradialytic hypotensive events were beyond the scope and resources of the trial but may have better explained the mechanisms of the reported benefits. Data to reliably inform the likely effect size were unavailable. With multiple imputation, the sample size retained 80% power to determine differences in the primary outcome of EF compared to the original target of 90%. The sample size also retained 90% power to resolve a 10% difference in LV peak systolic strain (45), although data to inform estimations for this more sensitive outcome were unavailable when the trial was designed. There was greater than anticipated attrition prior to the first CMR assessment that was not treatment related. Similar attrition from CMR assessment has been reported in recent clinical trials in HD patients using CMR (35). Such attrition might in part be due to recruiting from an incident population, who were adjusting to starting on HD and wide inclusion criteria allowing enrolment of patients with significant comorbidity that were less likely to undergo CMR.

**Conclusion**
Whilst the primary outcome of EF was unchanged, this study is the first to demonstrate that an intervention delivered within the context of conventional thrice weekly HD, attenuates the progression of cardiomyopathy by reducing LV mass and dilation and preserving LV strain and aortic distensibility without adverse events. Dialysate cooling was operationally simple to deliver, well tolerated, universally applicable and cost free. This strongly suggests that attention to the hemodynamic tolerability of dialysis is important to improve long term HD patient outcomes and warrants further definitive study or wider adoption.

Disclosures

All the authors declared no competing interests.

Acknowledgements

This study was funded by a National Institute for Health Research Research for Patient Benefit Grant (PB-PG-0408-16195). Dr. Odudu acknowledges the support of a British Heart Foundation Clinical Research Training Fellowship Grant (FS/11/10/28564) and a National Institute for Health Research Clinical Lectureship. Dr. McCann is supported by a National Institute for Health Research Post-Doctoral Research Fellowship (PDF-2011-04-51). The study was sponsored by Derby Hospitals NHS Foundation Trust. The funder had no role in the design and conduct of the study; the collection, management, analysis and interpretation of the data; or the preparation, review, or approval of the manuscript. We acknowledge the statistical support of Mr Apostolos Fakis. We acknowledge the use of software and analytic assistance of Dr Mark Horsfield. We thank Prof. Simon Davies, Dr. Veena Reddy, Dr. Indranil Dasgupta, study participants and nursing and allied healthcare professionals for their time. We thank the magnetic resonance radiographers led by Mrs. Kathryn Appleyard at the Nuffield Derby Hospital and Mr. David Capener at the University of Sheffield. We acknowledge useful discussions with Dr. Robert Foley and the assistance of Dr. James Fotheringham with data parsing. We acknowledge the helpful comments of the anonymous peer reviewers. Parts of the study outcomes were presented in a preliminary form at the American Society of Nephrology conference in November 2013.
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   myocardial strain and left ventricular twist measured using complementary spatial modulation of 
**Table 1 Baseline patient characteristics.**

Continuous data were tested for normality and summarized using mean±SD or median (interquartile range). Categorical data were expressed as counts [percentages]. Abbreviations; BMI= Body Mass Index; BSA=Body Surface Area; RAAS= Renin Angiotensin Aldosterone System; BSA= Body Surface Area; EF=ejection fraction; LV= left ventricle.

**Table 2 Trial outcomes**  
*Mean±SD of observed values were adjusted using a linear mixed-effects model for study center, age, sex and diabetes status.  
*Treatment effects are expressed as mean(95% CI) and were determined by the interaction of treatment group with time.  
*Systolic strain values are conventionally expressed on a negative scale as a percentage change in LV length from baseline, with more negative changes denoting better systolic function.  
*Systolic strain rate values are expressed on a negative scale with more negative changes denoting better systolic function.  
*Diastolic strain rate values are a measure of diastolic function with greater values denoting better function.  
*Aortic distensibility data were skewed and therefore used logarithmically transformed values, with coefficients back-transformed for presentation. LV= left ventricle, EF=left ventricular ejection fraction, BSA= Body Surface Area.
Figure 1 CMR analysis in a typical study participant. Tagged LV short-axis images (A), derived LV segmental strain curves (B). These were averaged to determine global LV strain (C) and strain-rate (D) curves from which peak-systolic and diastolic values were used as study outcomes (arrows). Aortic distensibility was determined by semi-automated tracing of ascending aorta in 30 phases per cardiac cycle (E) to derive aortic area (F, red line, left y axis) and aortic blood flow (F, blue line, right y axis).

Figure 2 Trial Consolidated Standards Of Reporting Flow Chart

Figure 3 Trial outcomes expressed as standardized effect sizes with 95% CI. The mean changes from Table 2 are divided by the pooled SD of the variable at baseline.

Figure 4 Within-group comparisons of adjusted mean±SE of absolute change in peak-systolic strain (%) from baseline to 12 months using the American Heart Association (AHA) 16 segment model of the LV. Strain values are conventionally expressed on a negative scale as a percentage change in length from baseline, with larger changes denoting better function. p-values are for the interaction of treatment group with time in a linear mixed-effects model. *p<0.05; **p<0.01.
Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control Dialysate temperature 37 C (n=28)</th>
<th>Individualised Cooled Dialysate temperature (n=26)</th>
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<tr>
<td>Female</td>
<td>60(25)</td>
<td>60(26)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28±6</td>
<td>28±6</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142(28)</td>
<td>140(28)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76±13</td>
<td>75±11</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>62(29)</td>
<td>59(30)</td>
</tr>
</tbody>
</table>

**Medical history**

HD vintage (days) 135±69 122±69
Tunelled catheter 8[29] 5[19]
Arteriovenous fistula 20[71] 21[81]
Diabetes mellitus 8[29] 7[27]
Ischemic heart disease 4[14] 3[12]
Current/Ex-smoker 8[36] 13[59]

**Primary renal disease**

Diabetes Mellitus 8[29] 6[21]
Unknown/Others 6[21] 4[14]

**Medication**

Treated hypertension 21[75] 21[81]
RAAS antagonist 8[29] 6[23]
Beta-blocker 9[32] 10[39]
Statin use 12[43] 11[42]
Calcium-containing 9[32] 5[19]
Non-calcium 9[32] 5[19]
Erythropoeisis Stimulating Agent 21[75] 26[69]
Vitamin D analogue 16[57] 15[58]

**Laboratory values**

Hemoglobin g/dl 10.9±1.4 11.1±1.6
Calcium (mmol/L) 2.3±0.2 2.3±0.1
Phosphate(mmol/L) 1.6(0.4) 1.5(0.9)
Albumin (mmol/L) 35.8±3.6 35.7±3.3
Total Cholesterol (mmol/L) 3.3(0.6) 3.6(0.8)
Ultrafiltration per session (L) 2.0±0.9 2.0±0.8
<table>
<thead>
<tr>
<th>Endpoint(^a)</th>
<th>Treatment</th>
<th>Baseline</th>
<th>12 months</th>
<th>Treatment difference between groups(^b)</th>
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<tbody>
<tr>
<td>EF (%)</td>
<td>control</td>
<td>58.7±8.5</td>
<td>57.6±8.5</td>
<td>1.5(-4.3,7.3)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>57.4±15.3</td>
<td>61.0±18.4</td>
<td></td>
</tr>
<tr>
<td>LV mass(g)</td>
<td>control</td>
<td>140.3±48.7</td>
<td>141.3±48.7</td>
<td>-15.6(-29.4,-1.9)</td>
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<tr>
<td></td>
<td>intervention</td>
<td>157.9±50.5</td>
<td>145.6±50.5</td>
<td></td>
</tr>
<tr>
<td>LV mass index/BSA (g/m(^2))</td>
<td>control</td>
<td>72.4±21.7</td>
<td>73.5±31.7</td>
<td>-8.1(-15.5,-0.8)</td>
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<tr>
<td></td>
<td>intervention</td>
<td>80.7±17.8</td>
<td>75.0±26.5</td>
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</tr>
<tr>
<td>Global peak-systolic strain (%)(^c)</td>
<td>control</td>
<td>-16.3±3.7</td>
<td>-13.0±6.9</td>
<td>-3.3(-6.5,-0.2)</td>
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<tr>
<td></td>
<td>intervention</td>
<td>-15.9±0.9±4.6</td>
<td>-15.9±7.6</td>
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<tr>
<td>(^d)Global peak-systolic strain rate (s(^{-1}))</td>
<td>control</td>
<td>-0.97±0.2</td>
<td>-0.87±0.21</td>
<td>-0.2(-0.3,-0.08)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>-1.02±0.3</td>
<td>-1.03±0.25</td>
<td></td>
</tr>
<tr>
<td>(^e)Global peak-diastolic strain rate (s(^{-1}))</td>
<td>control</td>
<td>1.05±0.3</td>
<td>0.90±0.42</td>
<td>0.18(0.017,0.34)</td>
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<tr>
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<td>intervention</td>
<td>1.12±0.4</td>
<td>1.00±0.41</td>
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<tr>
<td>LV end-diastolic volume (ml)</td>
<td>control</td>
<td>150.3±37</td>
<td>156.1±37</td>
<td>-23.8(-44.7,-2.9)</td>
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<td></td>
<td>intervention</td>
<td>162.9±39.8</td>
<td>144.5±39.8</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume index/ BSA (ml/m(^2))</td>
<td>control</td>
<td>78.8±21.2</td>
<td>75.6±31.2</td>
<td>-12.4(-23.2,-1.5)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>83.5±27</td>
<td>73.3±29.1</td>
<td></td>
</tr>
<tr>
<td>(^f)Aortic Distensibility (mmHg(^{-1})×10(^{-3}))</td>
<td>control</td>
<td>4.4±2.6</td>
<td>2.1±2.6</td>
<td>1.8(0.1,3.6)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>3.4±2.5</td>
<td>3.1±2.5</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

A

B

Segmental LV strain versus time

Segmental peak-systolic strains

0 250 500 750 1000
Time (ms)

C

D

Global LV strain versus time

Global peak-systolic strain

Global LV strain rate versus time

peak-diastolic strain rate

0 250 500 750 1000
Time (ms)

E

F

Aortic area (mm²)

Aortic Flow (mL/s)

0 100 200 300 400 500 600
Time (ms)
Assessed for eligibility (n=223)
• 29 did not meet inclusion criteria
• 121 declined to participate (inconvenient, adjusting to being on HD or disinterested (n=121).

Randomized (n=73)

Control dialysate temperature (37°C)
• 3 did not receive allocated intervention (1 withdrawal of consent for the trial, 1 relocation, 1 intercurrent illness)
• 6 received allocated intervention but did not have baseline CMR (4 did not tolerate CMR, 1 too obese for CMR, 1 unable to verify safety for CMR).

Intervention cooled dialysate (0.5°C below core body temperature)
• 5 did not receive allocated intervention (1 death, 1 pregnancy, 1 intercurrent illness, 2 withdrawal of consent for the trial).
• 5 received allocated intervention but did not have baseline CMR (1 death, 1 did not tolerate CMR, 2 withdrew consent for CMR, 1 too obese for CMR).

Analysed (n=28)
• 23 completed follow-up with 12 month CMR
• 5 required imputation for missing 12 month CMR data (1 death, 2 renal transplantation, 2 declined 12 month CMR).

Analysed (n=26)
• 21 completed follow-up with 12 month CMR
• 5 required imputation for missing 12 month CMR (3 renal transplantation, 1 pacemaker insertion during study period, 1 declined 12 month CMR).
Figure 3

Estimated Standardized Treatment Effect (95%CI)

- LVEF
- LV mass
- LV mass index
- Peak-systolic strain
- Peak-systolic strain rate
- Peak-diastolic strain rate
- LV end-diastolic volume
- LV end-diastolic volume index
- Aortic distensibility

Standard-Deviation Units

Standard dialysate better  Cooler dialysate better
Figure 4

Adjusted mean change in segmental peak-systolic strain

Control
Cooled

Δsegmental peak-systolic strain(%)
Supplemental materials to the manuscript: Randomized controlled trial of individualized dialysate cooling for cardiac protection in hemodialysis patients by Odudu et al.

Supplemental Methods:

Follow-up data imputation

As per the intention-to-treat principle, follow-up CMR data were imputed for 10 patients to avoid the biased treatment outcome estimates that are associated with listwise deletion of study dropouts (1). With the exception of younger age, the intention-to-treat cohort was not significantly different from the complete-case cohort (Supplemental Table 1). Multiple imputations used Markov Chain Monte Carlo estimation methods available in SPSS version 21. Twelve simulated data sets were created with 10,000 iterations to derive pooled parameter estimates using Rubin’s rules (2). The variables used for the imputation were study centre, age, sex, smoking history, age, diabetes status, ischemic heart disease status, vascular access and all baseline observed variables. The complete-case analysis did not change our findings (Supplemental Table 2).
Supplemental Table 1: Baseline characteristics stratified by study completion.  
Abbreviations; RAAS= Renin Angiotensin Aldosterone System.

<table>
<thead>
<tr>
<th></th>
<th>Completed the Study (n=44)</th>
<th>Did not complete the study (n=10)</th>
<th>P val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>59(22)</td>
<td>49(28)</td>
<td>0.03</td>
</tr>
<tr>
<td>Female (%)</td>
<td>13(30)</td>
<td>2(20)</td>
<td>0.7</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>29±6</td>
<td>25±4</td>
<td>0.2</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140(27)</td>
<td>144(61)</td>
<td>0.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74(17)</td>
<td>87(22)</td>
<td>0.08</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>62(30)</td>
<td>56(39)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD vintage (d)</td>
<td>135±69</td>
<td>122±69</td>
<td>0.8</td>
</tr>
<tr>
<td>Tunnelled catheter (%)</td>
<td>12(27)</td>
<td>2(20)</td>
<td>1</td>
</tr>
<tr>
<td>Arteriovenous fistula (%)</td>
<td>32(73)</td>
<td>8(80)</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>12(27)</td>
<td>2(20)</td>
<td>1</td>
</tr>
<tr>
<td>Ischemic heart disease (%)</td>
<td>4(9)</td>
<td>3(40)</td>
<td>0.1</td>
</tr>
<tr>
<td>Current/Ex-smoker (%)</td>
<td>21(48)</td>
<td>4(40)</td>
<td>0.7</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>7(16)</td>
<td>0(0)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated hypertension (%)</td>
<td>35(80)</td>
<td>7(10)</td>
<td>0.7</td>
</tr>
<tr>
<td>RAAS antagonist (%)</td>
<td>11(25)</td>
<td>3(30)</td>
<td>0.7</td>
</tr>
<tr>
<td>β-blocker (%)</td>
<td>15(34)</td>
<td>4(40)</td>
<td>0.7</td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>20(46)</td>
<td>3(30)</td>
<td>0.5</td>
</tr>
<tr>
<td>Phosphate binder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium-containing (%)</td>
<td>13(30)</td>
<td>2(20)</td>
<td>0.7</td>
</tr>
<tr>
<td>Non-calcium (%)</td>
<td>12(27)</td>
<td>2(20)</td>
<td>1</td>
</tr>
<tr>
<td>Erythropoiesis Stimulating Agent (%)</td>
<td>31(71)</td>
<td>8(80)</td>
<td>0.7</td>
</tr>
<tr>
<td>Vitamin D analogue (%)</td>
<td>24(55)</td>
<td>7(70)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Laboratory values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin g/dl</td>
<td>11.0±1.6</td>
<td>12.3±0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.2±0.8</td>
<td>9.6±0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Phosphate (mg/dl)</td>
<td>4.6(1.5)</td>
<td>4.3 (1.5)</td>
<td>0.8</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.6±0.3</td>
<td>3.8±0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>135.1(27)</td>
<td>158.3(34.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Ultrafiltration per session (L)</td>
<td>2.0±0.8</td>
<td>2.2±1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Supplemental Table 3: Trial outcomes in the complete-case cohort with no missing data (n=44, 23 control, 21 intervention). Table annotations are identical to Table 2 in the main manuscript.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Treatment</th>
<th>Baseline</th>
<th>12 months</th>
<th>Treatment difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>control</td>
<td>59.5±6.7</td>
<td>61±11</td>
<td>0.7(-5.5,6.9)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>60±13.7</td>
<td>62.3±11</td>
<td></td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>control</td>
<td>143.9±58</td>
<td>147.6±58</td>
<td>-15.5(-28.9, -2.1)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>160.1±44.9</td>
<td>148.3±39.4</td>
<td></td>
</tr>
<tr>
<td>LV mass indexed to BSA (g/m²)</td>
<td>control</td>
<td>73±23.5</td>
<td>76±23.5</td>
<td>-8.2(-15.4, -1.1)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>81.9±17.4</td>
<td>76.6±17.4</td>
<td></td>
</tr>
<tr>
<td>Global peak-systolic strain (%)</td>
<td>control</td>
<td>-16.2±3.8</td>
<td>-13.3±17.7</td>
<td>-2.6(-4.6, -0.5)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>-15.9±4.6</td>
<td>-15.6±5</td>
<td></td>
</tr>
<tr>
<td>Global peak-systolic strain rate (s⁻¹)</td>
<td>control</td>
<td>-0.99±0.2</td>
<td>-0.87±0.2</td>
<td>-0.22(-0.40, -0.04)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>-1.04±0.3</td>
<td>-1.01±0.2</td>
<td></td>
</tr>
<tr>
<td>Global peak-diastolic strain rate (s⁻¹)</td>
<td>control</td>
<td>1.01±0.2</td>
<td>0.86±0.3</td>
<td>0.23(0.03, 0.43)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>1.04±0.3</td>
<td>0.96±0.3</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume (ml)</td>
<td>control</td>
<td>146±45.1</td>
<td>147.3±40.8</td>
<td>-19.4(-38.2,-0.46)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>149.7±39.9</td>
<td>134.0±29.8</td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic volume indexed to BSA (ml/m²)</td>
<td>control</td>
<td>75.3±22.1</td>
<td>76.7±19.7</td>
<td>-9.4(-18.7,-0.03)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>76.9±18.3</td>
<td>69.7±16</td>
<td></td>
</tr>
<tr>
<td>Aortic Distensibility (mmHg⁻¹×10⁻³)</td>
<td>control</td>
<td>5.5±2.9</td>
<td>2.5±2.9</td>
<td>2.8(0.6,5.0)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>3.7±2.7</td>
<td>3.4±2.7</td>
<td></td>
</tr>
</tbody>
</table>
Post-hoc explanatory analyses

The intervention group had a non-significantly greater LV mass at baseline (Supplemental Table 3). The mixed-model accounted for baseline differences in a similar fashion to ANCOVA. However, to explore if the baseline LV mass modified the reported effects on LV mass, a linear regression of the treatment effect on baseline LV mass was made with natural cubic splines by three equally spaced knots as previously described (Supplemental Figure 1) (3). Higher baseline LV mass was modestly correlated to greater reductions in LV mass at follow-up (adjusted $R^2$ of 0.17, p=0.005) but the effects were not significantly different between groups (p value for the interaction 0.65). There were no significant between-group differences in body composition by bioimpedance (Supplemental Table 4), interdialytic weight gain or pre-dialysis mean arterial blood pressure (Supplemental Figures 2 and 3).

**Supplemental Table 3: Extended Baseline characteristics.** Values are mean±SD or median(interquartile range)

<table>
<thead>
<tr>
<th>CMR parameters</th>
<th>Control Dialysate temperature 37°C (n=28)</th>
<th>Individualised Cooled Dialysate temperature (n=26)</th>
<th>p val</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>58.7±8.5</td>
<td>57.4±15.3</td>
<td>0.9</td>
</tr>
<tr>
<td>LV mass(g)</td>
<td>140.3±48.7</td>
<td>157.9±50.5</td>
<td>0.2</td>
</tr>
<tr>
<td>LV mass indexed to BSA (g/m$^2$)</td>
<td>72.4±21.7</td>
<td>80.7±17.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Global peak-systolic strain (%)</td>
<td>-16.3±3.7</td>
<td>-15.9±0.9±4.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Global peak-systolic strain rate (1/s)</td>
<td>-0.97±0.2</td>
<td>-1.02±0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Global peak-diastolic strain rate (1/s)</td>
<td>1.05±0.3</td>
<td>1.12±0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>LV end-diastolic volume (ml)</td>
<td>150.3±37</td>
<td>162.9±39.8</td>
<td>0.3</td>
</tr>
<tr>
<td>LV end-diastolic volume indexed to BSA (ml/m$^2$)</td>
<td>78.8±21.2</td>
<td>83.5±27</td>
<td>0.5</td>
</tr>
<tr>
<td>Aortic Distensibility (mmHg$^{-1}$×10$^{-3}$)</td>
<td>2.6(3.3)</td>
<td>2.6(1.5)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
**Supplemental Figure 1:** The effect of baseline left ventricular mass on the change in left ventricular mass in the dialysate cooling trial. A linear regression with a cubic spline and 3 equally spaced knots of mean and 95% CI of treatment effect on baseline LV mass (adjusted $R^2$ of 0.17, $p=0.005$). There was no significant difference between treatment groups ($p$ value for the interaction 0.65).

![Graph showing the treatment effect on LV mass by level of baseline LV mass](image)

**Supplemental Table 1** Body volume status by multiple frequency bioimpedance in the dialysate cooling trial. Treatment differences are mean(95%CI)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
<th>Baseline</th>
<th>12 months</th>
<th>Treatment difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body Water (L)</td>
<td>control</td>
<td>41.3±6.1</td>
<td>40.6±6.2</td>
<td>0.4(-1.7,2.5)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>44±5.6</td>
<td>43.7±5.9</td>
<td></td>
</tr>
<tr>
<td>Extracellular Water (L)</td>
<td>control</td>
<td>16.2±2.7</td>
<td>16.0±2.7</td>
<td>0(-0.8,0.8)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>17.7±2.5</td>
<td>17.5±2.6</td>
<td></td>
</tr>
<tr>
<td>Intracellular Water (L)</td>
<td>control</td>
<td>25.1±3.7</td>
<td>24.6±3.8</td>
<td>0.4(-0.9,1.6)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>26.2±3.5</td>
<td>26.2±3.6</td>
<td></td>
</tr>
<tr>
<td>Extracellular Water/Total Body Water</td>
<td>control</td>
<td>0.39±0.02</td>
<td>0.39±0.02</td>
<td>0(0,0)</td>
</tr>
<tr>
<td></td>
<td>intervention</td>
<td>0.40±0.02</td>
<td>0.40±0.02</td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Figure 2: Repeated measures of pre-dialysis mean arterial blood pressure by treatment group. The red and green lines and their respective shaded bands lines represent adjusted means and standard error by locally weighted-regression (loess) for the control and intervention group respectively across the study period. A linear mixed model showed no significant between group differences with time.

Supplemental Figure 3: Repeated measures of inter-dialytic weight gain by treatment group. The red and green lines and their respective shaded bands represent the adjusted means and standard error by locally weighted-regression (loess) for the control and intervention group respectively. A linear mixed model showed no significant between group differences with time.
References

