A Randomized Multicenter Open Label Blinded End Point Trial Comparing the Effects of Spironolactone to Chlorthalidone on Left Ventricular Mass in Patients with Early Stage Chronic Kidney Disease: Rationale and Design of the SPIRO-CKD trial

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1. Title

A Randomized Multicenter Open Label Blinded End Point Trial Comparing the Effects of Spironolactone to Chlorthalidone on Left Ventricular Mass in Patients with Early Stage Chronic Kidney Disease: Rationale and Design of the SPIRO-CKD trial.

EudraCT No. 2013-002636-25, current controlled trials: ISRCTN 94696478

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2. Abstract

**Background:** Chronic kidney disease (CKD) is associated with increased left ventricular (LV) mass and arterial stiffness. In a previous trial, spironolactone improved these endpoints compared to placebo in subjects with early stage CKD, but it is not known whether these effects were specific to the drug or secondary to blood pressure lowering.

**Aim:** To investigate the hypothesis that spironolactone is superior to chlorthalidone in the reduction of LV mass while exerting similar effects on blood pressure.

**Design:** This is a multi-center, prospective, randomized open-label blinded endpoint (PROBE) clinical trial initially designed to compare the effects of 40 weeks of treatment with spironolactone 25 mg once daily to chlorthalidone 25 mg once daily on the co-primary endpoints of change in pulse wave velocity and change in LV mass in 350 patients with stages 2 and 3 CKD on established treatment with an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker. Due to slow recruitment rates it became apparent that it would not be possible to recruit this sample size within the funded time period. The study design was therefore changed to one with a single primary end-point of LV mass requiring 150 patients. Recruitment was completed on 31st December 2016 at which time 154 patients had been recruited. Investigations included cardiac magnetic resonance imaging, applanation tonometry, 24-hour ambulatory blood pressure monitoring, and laboratory tests. Subjects are assessed before and after 40 weeks of randomly allocated drug therapy and at 46 weeks after discontinuation of the study drug.

Trial Registration: This trial is registered with the European Union Drug Regulating Authorities Clinical Trials: **EudraCT No. 2013-002636-25** and Current Controlled Trials: **ISRCTN 94696478. UK Medicines and Healthcare Regulatory Agency (MHRA) Clinical Trials Authorization No. 21761/0295/001-0001**

**Keywords**
Chronic kidney disease, left ventricular mass, left ventricular hypertrophy, arterial stiffness, mineralocorticoid receptor blockers, aldosterone antagonists, spironolactone

3. Main Text

Background

Levels of cardiovascular mortality and morbidity are high in chronic kidney disease (CKD) with a graded inverse relationship with glomerular filtration rate. This has a large public health impact because of the high prevalence of early stage CKD which affects about 1 in 7 people in the US. Although patients with CKD have a clustering of atherosclerotic risk factors, the majority of deaths are due to heart failure and arrhythmia. This suggests that the underlying mechanism for heart disease in patients with CKD is not coronary atherosclerosis but myocardial disease, so called ‘uremic cardiomyopathy’. In the early stages of CKD, sensitive markers of systolic (strain) and diastolic function are often abnormal and there is an increased prevalence of left ventricular hypertrophy (LVH). Causative factors are thought to include increased arterial stiffness, hypertension, sympathetic neural influences and circulating factors including mediators of the renin–angiotensin-aldosterone system. These factors are already present in stage 2 to 3 CKD and provide potential targets for treatment aimed at preventing the development and progression of myocardial hypertrophy and fibrosis.

Aldosterone is a key mediator of cardiovascular disease in many conditions including heart failure and CKD. This hormone causes vascular and myocardial injury and fibrosis, particularly in the presence of sodium excess as occurs in CKD. Mineralocorticoid receptor antagonists ameliorate these actions in cellular and animal models and improve clinical outcomes in people with heart failure. Like heart failure, CKD is characterized by sodium overload and high aldosterone concentrations due to aldosterone escape (unsuppressed levels of aldosterone despite chronic sodium overload) and aldosterone breakthrough (high circulating aldosterone levels despite suppression with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blocker (ARB) drugs). In the
CRIB-2 trial of 112 patients with stages 2 and 3 CKD, we showed that compared to placebo, spironolactone reduced LV mass, arterial stiffness and collagen turnover and improved myocardial diastolic function. (9, 10) As blood pressure was also reduced by spironolactone with a mean fall in systolic pressure of 11 mmHg, it is possible that the effects of spironolactone on cardiovascular structure and function were non-specific and mediated by blood pressure lowering alone. To address whether the benefits of spironolactone in CRIB-2 were specific to mineralocorticoid blockade or as a result of a reduction in blood pressure we designed the Spironolactone in Chronic Kidney Disease (SPIRO-CKD) trial to include an active control drug to lower blood pressure to similar levels. Chlorthalidone was chosen as the control drug due to its proven anti-hypertensive action in patients with early stage CKD with an effect size in the ALLHAT sub-study in this patient group about equal to those of spironolactone in CRIB-2. (11) The SPIRO-CKD trial was initially designed to investigate the hypothesis that spironolactone is superior to chlorthalidone in the reduction of a co-primary end point consisting of LV mass and arterial stiffness. Recruitment was slower than anticipated due to smaller numbers of patients with early stage CKD being reviewed routinely in a number of participating centers. Following discussions with the British Heart Foundation (funding body) and the Trial Steering Committee it was decided that it would not be possible to recruit a sample size sufficient to provide adequate statistical power to detect a change in arterial stiffness. The study design has therefore changed to one with a single primary end point of LV mass.

Methods

Study design

This multi-center, prospective, randomized open-label blinded endpoint (PROBE) clinical trial was initially designed to compare the effects of 40 weeks of treatment with spironolactone 25 mg once daily to chlorthalidone 25 mg once daily on the co-primary end point of change in LV mass using cardiac magnetic resonance imaging (MRI) and pulse wave velocity in patients with stages 2 and 3 CKD, without known cardiovascular disease or diabetes mellitus
and on established treatment with an ACE inhibitor or an ARB with controlled blood pressure on study entry. Nested sub-studies were also designed to compare the effects of these drugs on changes in LV systolic and diastolic deformation (global longitudinal strain) and in LV interstitial fibrosis (T1 mapping), both measured non-invasively by cardiac MRI. Calculation of estimated glomerular filtration rate (eGFR) has been performed using the 4 variable Modification of Diet in Renal Disease formula. CKD stages 2 and 3 have been defined as an eGFR of 60-89 ml/min/1.73m$^2$ in the presence of another abnormality e.g. albuminuria or a structural abnormality of the kidney, and 30-59 ml/min/1.73m$^2$ respectively. At the time of recruitment creatinine was measured from blood tests performed within the last 12 months, on 2 occasions, at least 90 days apart. Details of the inclusion and exclusion criteria, and the study outcome measures are listed in Tables 1 and 2 respectively. The academic centres participating in the trial will be the University of Birmingham, the University of Cambridge, University College London and the University of Edinburgh, UK. Patients have been recruited from the Queen Elizabeth Hospital Birmingham, Addenbrookes Hospital, Cambridge, the Royal Free Hospital, London, the Western General Hospital Edinburgh, and primary care practices in Edinburgh. The trial started recruitment in June 2014, and aimed to recruit 350 patients over a 2-year period. However, the rate of recruitment was slower than anticipated, and it became evident by November 2015 that recruitment of this number would not be completed within the funded time and the decision was taken to change the study design to one with the single primary end point of change in LV mass. This allowed a sample size which could be recruited within the funded time frame, (see sample size calculations section). This decision was made following discussions with the funder – the British Heart Foundation, the Trial Management Group and the Trial Steering Committee who remain blind to any interim data analyses.

Ethical approval for this study was awarded by the West Midlands National Research Ethics Service Committee in September 2013 (13/WM/0304). The study is funded by the British Heart Foundation (SP/12/8/2962) and is registered with Medicines and Healthcare products
Regulatory Agency (MHRA) (Clinical Trials Authorization No. 21761/0295/001-0001). The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.
Table 1: Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;18 years and willing to undergo investigations</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Chronic kidney disease stage 2 or 3 (eGFR 30-89 ml/min/1.73m²) from blood tests performed within the last 12 months, on two occasions, at least 90 days apart. (For stage 2 CKD, no criteria for urinary or blood test or structural abnormalities were mandated, these criterial were left to the discretion of the local principal investigator)</td>
<td>Clinical evidence of hypovolemia</td>
</tr>
<tr>
<td>Controlled blood pressure (no current indication for additional anti-hypertensive therapy in the opinion of the local principal investigator)</td>
<td>On current regular treatment with non-steroidal anti-inflammatory drugs, or other agents (except ACE inhibitors, ARBs, or low dose aspirin) that might cause a reduction in eGFR.</td>
</tr>
<tr>
<td>On established (&gt;6 weeks) treatment with ACE inhibitors or ARBs</td>
<td>Recent (&lt; 6 months) acute myocardial infarction or other major adverse cardiovascular event (STEMI*, NSTEMI**, unstable angina, coronary revascularization, stroke, transient ischemic attack)</td>
</tr>
<tr>
<td>Clinically stable (no hospital admission or other significant acute illness within 3 months, and no recent (&lt;6 months) acute myocardial infarction</td>
<td>Known left ventricular systolic dysfunction (ejection fraction &lt;50%) or severe valvular heart disease or evidence of heart failure</td>
</tr>
<tr>
<td>Females of childbearing potential must not be pregnant or breast feeding, and must agree to avoid pregnancy and to use adequate, medically approved contraceptive precautions during and for 6 weeks following the last dose of study treatment.</td>
<td>Active malignant disease with a life expectancy of &lt;5 years</td>
</tr>
<tr>
<td>Males with a partner of childbearing potential must agree to use medically approved contraception during and for 6 weeks following the last dose of study treatment.</td>
<td>Previous hyperkalemia (K+ ≥6.0 mmol/l) without precipitating cause</td>
</tr>
<tr>
<td>Serum K+ &gt; 5.0 at entry</td>
<td>Serum K+ &gt; 5.0 at entry</td>
</tr>
<tr>
<td>Serum sodium &lt;130 mmol/l at entry</td>
<td>Serum sodium &lt;130 mmol/l at entry</td>
</tr>
<tr>
<td>Atrial fibrillation on screening ECG</td>
<td>Current treatment with spironolactone or other mineralocorticoid receptor blocker</td>
</tr>
<tr>
<td>Use of a thiazide or loop diuretic in the 6 weeks prior to enrolment</td>
<td>Use of a thiazide or loop diuretic in the 6 weeks prior to enrolment</td>
</tr>
<tr>
<td>Pregnant or breastfeeding</td>
<td>Pregnant or breastfeeding</td>
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<tr>
<td>Known alcohol or drug abuse</td>
<td>Known alcohol or drug abuse</td>
</tr>
<tr>
<td>Active chronic diarrhea</td>
<td>Active chronic diarrhea</td>
</tr>
<tr>
<td>Recent active gout (within 3 months)</td>
<td>Recent active gout (within 3 months)</td>
</tr>
<tr>
<td>Acute kidney injury in previous 3 months</td>
<td>Acute kidney injury in previous 3 months</td>
</tr>
<tr>
<td>Documented Addison’s disease</td>
<td>Documented Addison’s disease</td>
</tr>
<tr>
<td>Treatment with fludrocortisone, co-trimoxazole or lithium</td>
<td>Treatment with fludrocortisone, co-trimoxazole or lithium</td>
</tr>
<tr>
<td>Combination treatment with ACE inhibitor and ARB</td>
<td>Combination treatment with ACE inhibitor and ARB</td>
</tr>
<tr>
<td>Office blood pressure &lt;115 mmHg systolic or &lt;50 mmHg diastolic</td>
<td>Office blood pressure uncontrolled and requiring urgent non-trial treatment</td>
</tr>
<tr>
<td>Office blood pressure uncontrolled and requiring urgent non-trial treatment</td>
<td>Unable to provide informed consent</td>
</tr>
</tbody>
</table>

*STEMI = ST-segment elevation myocardial infarction |
**NSTEMI = non-ST-segment elevation myocardial infarction
**STEMI, ST elevation myocardial infarction; **NSTEMI, non-ST elevation myocardial infarction.

Table 2: Primary and secondary outcome measures

<table>
<thead>
<tr>
<th>Primary outcome measures</th>
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<tr>
<td>Change between baseline and 40 weeks in LV mass measured by cardiac magnetic resonance imaging</td>
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</table>

<table>
<thead>
<tr>
<th>Secondary outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change between baseline and 40 weeks in arterial stiffness measured by carotid-femoral pulse wave velocity.</td>
</tr>
<tr>
<td>Change between baseline and 40 weeks in office, central and ambulatory blood pressures.</td>
</tr>
<tr>
<td>Change between baseline and 40 weeks in urinary albumin:creatinine ratio.</td>
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<tr>
<td>Changes between baseline and 40 weeks in left ventricular volumes and systolic function.</td>
</tr>
<tr>
<td>Changes between baseline and 40 weeks in plasma NT-pro-BNP.</td>
</tr>
<tr>
<td>Incidence of hyperkalaemia</td>
</tr>
<tr>
<td>Change between baseline and 40 weeks in eGFR</td>
</tr>
</tbody>
</table>
Recruitment and Screening

Recruitment of 154 patients was completed by 31st December 2016. Eligible patients (Table 1) were identified using hospital and primary care medical records. All recruitment took place within hospital outpatient clinics in the aforementioned institutions in two stages. Firstly, all patients apparently fulfilling the eligibility criteria were asked for written consent for additional screening procedures to be undertaken. Screening procedures were:

1) Ensuring potential participants met the study’s inclusion and exclusion criteria.

2) Physical examination to check pulse, office blood pressure, signs of hypovolemia and a cardiovascular exam to exclude signs of heart failure or valvular disease.

   Echocardiography was performed only in cases of clinical uncertainty about the presence of heart failure or valve disease.

3) Recording an electrocardiogram (EKG).

4) Blood tests for routine biochemical and hematological parameters including eGFR.

5) Females of child-bearing potential underwent a pregnancy test.

When patients fulfilled the eligibility criteria, informed written consent was obtained for randomization into the main SPIRO-CKD trial in keeping with the principles set out by the Declaration of Helsinki.

Run-in Phase

For those patients wanting to take part but not immediately eligible because they are either on existing diuretic therapy or not taking ACE inhibitor or ARB therapy or taking both of these agents, a 4-6 week run-in phase is utilized. For patients on existing loop or thiazide diuretic therapy who wish to take part in the trial; after obtaining consent diuretic therapy is stopped and the patient reviewed after 4 weeks to ensure he/she meets the eligibility criteria (table 1). For patients not on ACE inhibitor or ARB therapy, after obtaining consent, an ACE inhibitor or ARB of the physician’s choice is introduced and titrated to a well-tolerated therapeutic dose over a 4 week period. The patient is then reviewed to ensure eligibility. For patients on dual ACE inhibitor and ARB therapy, after obtaining consent, either the ACE
inhibitor or the ARB is stopped. Patients are then reviewed to ensure eligibility. Those patients who fulfill all entry criteria are consented and randomized into the main SPIRO-CKD trial.

**Randomization**

Once informed consent is received and the baseline assessments completed, patients are entered into the main SPIRO-CKD trial. Participants are randomized in a one-to-one ratio to either spironolactone (25 mg once a day) or chlorthalidone (half a 50 mg tablet once a day as there are no 25 mg tablets available for use in the UK) for 40 weeks without blinding. A secure central randomization service was provided by the Birmingham Clinical Trials Unit (BCTU, University of Birmingham) using a computer-generated program, using a minimization algorithm to ensure balance between the arms with regards to the important clinical variables of blood pressure, age and gender. Compliance with treatment is monitored by study coordinators using tablet counting.

**Investigations**

The trial design and schedule of investigations are summarized in figure 1 and further information is available in appendix 1. All baseline investigations have now been completed, and patients are being followed as per the study schedule. The trial consists of a 40 week period of randomized treatment followed by a 6 week period designed to detect effects caused by prolonged treatment rather than direct pharmacological effects. At the baseline visit, informed consent is taken, and weight and heart rate are recorded. Office blood pressure is measured using a British Hypertension Society validated semi-automated device on 3 occasions in the seated position after 5 minutes of rest with research staff present during the measurement; the mean of the last 2 readings is used for analysis. Participants undergo 24-hour ambulatory blood pressure monitoring at baseline using a British Hypertension Society validated oscillometric recorder set to make measurements every 30 minutes between 08.00 and 22.00 and hourly during the remaining hours. Additionally, all
patients undergo blood and urine tests, a cardiac MRI scan, pulse wave velocity and pulse wave analysis testing, after which the trial medication is given to the patient. More detail on the study investigations is given below. The measurement and analysis of end points is performed by observers blinded to treatment allocation.

Participants are followed-up at weeks 1, 2, 4, 8, 24, 40 and 46 post-randomization. In the event of participants being unable to attend for scheduled visits due to other inter-current issues, they are asked to attend at the closest possible date, whenever possible within 2 weeks of the due date. Where a participant cannot attend their week 40 study visit and investigations, they are asked to either undertake this visit up to 3 weeks before the due date, or alternatively to continue the study medication until the scheduled investigations can be undertaken (whenever possible within 4 weeks of the planned date).

The investigations listed below are carried out at time intervals during the trial as listed in figure 1 and appendix 1. All end points including Sphygmocor and CMR values are measured by investigators blinded to treatment allocation.

**Blood and urine:** Routine hematological and biochemical parameters, including plasma lipids, are recorded at weeks 0, 1, 2, 4, 8, 24, 40 and 46 as indicated in appendix 1. Plasma is stored for measurement assays of NT-pro-BNP and other biomarkers. Separate consent is also sought to take blood for DNA extraction to allow future analysis for genetic influences on kidney disease and response to treatment. Urine samples are collected for analysis of albumin:creatinine ratio and other biomarkers.

**Pulse wave velocity (PWV):** Sphygmocor (AtCor Medical, Sydney, Australia) studies are performed at weeks 0, 4, 24, 40 and 46. Carotid femoral PWV is the current standard technique for measuring aortic stiffness. (13) Subjects are studied in a quiet, temperature controlled room after 15 minutes of lying supine. Firstly, supine blood pressure is measured 3 times in the non-dominant arm, and the final 2 readings are averaged and entered into the machine software. Pulse Wave Velocity (SphygmoCor;) is determined by sequential acquisition of pressure waveforms from the carotid and femoral arteries by using a high-
fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX).\(^\text{(14, 15)}\) The path length is calculated by subtracting the distance between the sternal notch and the carotid recording site from the distance between the sternal notch and the femoral site.\(^\text{(13)}\)

**Pulse wave analysis:** Participants are requested to rest in a supine position for 5 minutes before measuring seated blood pressure 3 times in the non-dominant arm. Again an average of the final 2 blood pressures is entered into the machine software prior to undertaking applanation tonometry to record high-fidelity arterial pressure waveforms, from which indices relating to large artery stiffness can be calculated.\(^\text{(14, 16)}\) A micromanometer is used to flatten but not occlude the radial artery of the non-dominant arm using gentle pressure. An averaged peripheral waveform and corresponding central waveform is generated after 11 seconds of data capture. The central waveform will then be analyzed to determine the augmentation index (the difference between the second and first peaks of the central pressure waveform, expressed as a percentage of the pulse pressure) and central aortic pressures. This method has been shown to be reproducible in both healthy subjects and in patients with CKD.\(^\text{(14, 16)}\)

**Cardiac magnetic resonance imaging:** Cardiac magnetic resonance imaging is performed using standard clinical 1.5T (Siemens Aera and Avanto – London, Birmingham; GE Discovery MR450 - Cambridge), or 3T (Siemens Verio – Edinburgh) scanners at each site at weeks 0 and 40.

Image acquisition: The standard protocol is estimated to take 30 minutes. Serial contiguous short-axis cines are piloted from the vertical long-axis and horizontal short-axis images of the left and right ventricles for assessment of dimensions, volumes, LV function (ejection fraction, deformation) and mass in line with the standard cardiovascular MRI protocol.\(^\text{(17, 18)}\). Regional aortic distensibility will be assessed on cine imaging in the proximal ascending aorta using the formula: aortic distensibility=\(\Delta\)aortic area/(minimum aortic area×pulse pressure). Peripheral blood pressure is performed synchronously in triplicate at the brachial artery at the time of scanning for determination of pulse pressure.
Subjects are also asked for consent to participate in additional cardiac MRI sub-studies (detailed below). Participation in both sub-studies lengthens the cardiac MRI scan duration to approximately 40 and 50 minutes respectively.

**Sub-study A: Tagging (CSPAMM).** Cardiac MRI is used to assess regional myocardial function (deformation) and is the recognized reference standard for measuring strain. This sensitive measure of regional and global left ventricular contractile function permits the early detection of subtle left ventricular dysfunction which precedes decreases in ejection fraction.(19-21) Cine Spatial Modulation of Magnetization (CSPAMM) is used to generate a uniform grid pattern with 8-mm tag separation on the LV myocardium at 3 short-axis sections (basal, mid and apex) and the horizontal long axis image using a fast filed echo multishot sequence (minimum number of 15 phases per cardiac cycle) with prospective electrocardiogram gating.

**Sub-study B. T1 mapping.** T1 mapping using Modified Look Locker Inversion recovery sequence (MOLLI) is performed to characterize the myocardial tissue and quantify the extent of diffuse interstitial fibrosis.(22, 23) Pixel-based T1 maps will be reconstructed using inline motion correction in the LV horizontal long axis, basal and mid short axis slices will be acquired before and 15 minutes after contrast administration for myocardial T1 times and calculation of extracellular volume (ECV) using, a 5(3)3 sampling protocol- (average breath hold 10-15 seconds). A single, weight-adjusted dose (0.15 mmol/Kg) of non-ionic, macrocyclic intravenous gadolinium contrast (Gadavist®, Bayer Healthcare, Berkshire, England) is given to assess coarse irreversible fibrosis (late gadolinium enhancement) using standard inversion recovery imaging. In the event of a fall in eGFR to < 30 ml/min/1.73m², gadolinium contrast will not be administered on safety grounds.

Image analysis: Analysis of ventricular volumes, function and mass is performed offline using cvi42® software (Circle Cardiovascular Imaging, Calgary, Canada) at a central cardiac MRI lab by a single experienced clinician blinded to all trial data. Measurement of aortic cross-sectional area in systole and diastole is assessed using automated software (Matlab
6.5©, MathWorks Inc., Massachusetts, USA). (24) Measurement of aortic distensibility using cardiac MRI has low intra-observer variability and good reproducibility. (25) Tagging analysis is performed using CIMTag2D; University of Auckland, New Zealand and Tissue Tracking, cvi42® for LV strain, strain rate, and torsion calculation. (33) For T1 mapping, myocardial and blood relaxation times pre- and 15 minutes after gadolinium contrast are measured offline to calculate native T1 times and ECV using cvi42® software. (26) Segmental and global T1 values are obtained using the AHA 6 segment model in short axis slices and a manual region of interest in the basal septum from the 4-chamber image. Meticulous care will be taken to avoid the blood-myocardial boundary (10% offset both from the epicardial and endocardial borders) and any areas of LGE. Extracellular volume will be calculated using myocardial and blood T1 values before and after contrast using validated formulae. (27)

\[
ECV = \lambda \times (1 - Hct)
\]

Hct refers to the haematocrit recorded on a venous blood sample at the time of scan, \( \Delta R1 = 1/T1 \text{ time post contrast} - 1/T1 \text{ time pre contrast} \). Lambda (\( \lambda \)) refers to \((1/ T1 \text{ myocardium post contrast} - 1/T1 \text{ myocardium pre-contrast})/ (1/ T1 \text{ blood post contrast} - 1/ T1 \text{ blood pre-contrast})\). Normal reference ranges for T1 and ECV (mean ± 2 standard deviations) are defined using the healthy volunteers from a previous published study. (28)
Figure 1. Trial Procedure Flowchart

Identify potential participants and send a screening participation information sheet.

Informed consent for screening procedures to confirm eligibility.

Screening Procedures
- Clinical History
- Physical Examination
- Clinical Investigations

Run-in Phase for patients fulfilling entry criteria other than drug therapy.

Obtain informed consent then complete baseline investigations:
- blood tests, Sphygmocor, CMR, ABPM, biomarkers, BP and weight

Randomization to either Spironolactone 25mg od or Chlorthalidone ¼ of a 50mg tablet od for 40 weeks

Clinic visits at: 1, 2, 4, 8 and 24 weeks
- Perform blood tests and blood pressure
- Measures to ensure equality of BP response between treatment and placebo arms
- Drug compliance and adverse event monitoring

Clinic visit at week 40
- Repeat baseline assessments:
  - blood tests, Sphygmocor, CMR, ABPM, biomarkers, BP and weight
  - Drug compliance (tablet count) and adverse event monitoring

Week 46
- Blood tests, BP, weight
- Biomarkers
- Sphygmocor
- Adverse events evaluation
Withdrawal

Patients may withdraw consent from the study at any time. Patients may also withdraw from trial treatment, but continue with study follow-up and data collection as per the protocol. If the withdrawal is initiated by a healthcare professional, full details for the reason for withdrawal is recorded on the case report forms. In all other cases a simple statement reflecting patient preference is noted.

Pharmacovigilance

This trial is categorized by the Medicines and Healthcare products Regulatory Agency (MHRA) as type A (no higher than the risk of standard medical care). Patients are being followed up closely throughout this study, and an on-going evaluation of risk will continue throughout the recruitment period. Both spironolactone and chlorthalidone are considered investigational medicinal products (IMPs) within SPIRO-CKD trial. Any adverse events or serious adverse events that occur during this trial are reportable to the SPIRO-CKD Trials Office at the trials unit up to 6 weeks following last administration of the IMP. Any suspected unexpected serious adverse reactions related to the IMP will be reported irrespective of how long after IMP administration the reaction occurred. Responses to abnormalities of serum sodium, potassium and eGFR will be guided by pre-determined management plans (see appendix 2).

Blood pressure monitoring

In order to confirm or deny the trial hypothesis, it is essential that the change in blood pressure during the treatment period is not significantly different in each arm of the trial. A tight blood pressure target was thought impractical; instead, a blood pressure monitoring committee (BPMC) reviews the blood pressure data after each block of 20 patients for the first 100 patients, and then after each block of 30 patients to the end of trial recruitment. The precise frequency of review may change on the advice of the BPMC following their independent review of the data. Patients enter the trial with controlled blood pressure so that
there are not anticipated to be clinical reasons for adding further drugs for blood pressure control during the randomized treatment phase. In the event of a difference in blood pressure change becoming evident, the BPMC will advise on changes to medication in either arm.

**Data Monitoring Committee (DMC)**

An independent DMC has been convened for the trial. Interim analyses of major outcome measures and safety data are conducted and provided in strict confidence to the DMC. Any decision to stop the trial early will be based on the balance of efficacy and safety.

The DMC advises the chair of the Trial Steering Committee (TSC) if, in their view, any of the randomized comparisons in the trial have provided both: a) proof beyond reasonable doubt that for all, or for some, types of participant one particular intervention is definitely indicated or definitely contra-indicated in terms of a net difference of the major endpoint, and b) evidence that might reasonably be expected to influence the patient management of many clinicians who are already aware of the other main trial results.

Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but a difference of at least $p<0.001$ (similar to a Haybittle-Peto stopping boundary) in an interim analysis of a major endpoint may be required to justify halting, or modifying, the study prematurely. If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, so no fixed schedule is proposed. Given these relatively strict stopping criteria, no adjustment for multiple testing (to control the overall type I error rate) is proposed.

**Sample Size**

Data from the CRIB-2 study has been used to inform the sample size.(9) The standard deviation of change in arterial stiffness measured by PWV was 1.0 m/s in the spironolactone group and 0.9 m/s in the placebo group (with a mean difference between groups of 0.7 m/s). Using the larger of these standard deviations (1.0 m/s) for the sample size, to detect a
minimum relevant difference (MRD) of 0.4 m/s (the smallest difference that is of clinical significance and that can be detected within the limits of accuracy of the equipment used) in PWV (with 90% power, two sided alpha=0.025) required 157 per arm. Allowing for 10% drop-out, this required recruitment of 350 participants. The standard deviation of change in LV mass was 13 g in the spironolactone group and 11 g in the placebo group (with a mean difference between groups of 17 g). Again using the larger of these standard deviations (13 g) for the sample size calculation, to detect a MRD of 7 g in LV mass (with 90% power, two sided alpha=0.025) required 87 per arm. Allowing for 10% drop-out, this required recruitment of 200 participants.

The initial design of the study used a co-primary end point of change in LV mass and change in pulse wave velocity. A sample size of 350 patients was planned to give 90% power to detect a difference in PWV and >90% power to detect a change in LV mass with a p value of 0.025. When it became evident that this sample size was not achievable within the funded time, the primary outcome of the study was changed to the single end point of change in LV mass again using a MRD of 7g. With a two sided p value of 0.05 and a power of 85%, it was calculated that 63 patients per group would be required. Allowing for a 15% drop-out and missing data, this requires 150 participants.

**Statistical Analysis**

The primary comparison groups are composed of those randomized to spironolactone and those randomized to chlorthalidone. All analyses will be based on the intention to treat principle, with all patients analyzed in the treatment groups to which they were allocated irrespective of compliance with the randomized allocated treatment, and all patients will be included in the analyses. For all tests, summary statistics (e.g. mean differences, relative risks) will be reported and 95% confidence intervals will be constructed where appropriate. A p-value of <0.05 will be considered statistically significant, and there will be no adjustment for multiple comparisons.
Primary outcome analysis:
LV mass is measured at baseline and 40 weeks. Any missing cardiac MR data will be counted as missing in the first instance. A regression model with LV mass at 40 weeks as the outcome variable, and treatment group and baseline LV mass included as covariates in the model will be fitted. Results will be presented as a mean difference between groups with a 95% confidence interval. These analyses assume that the data will be normally distributed (as is expected). Details of non-parametric analysis methods that will be used should the assumption of normality not hold will be provided in the Statistical Analysis Plan.

Secondary outcome analysis:
Arterial stiffness is measured at baseline, and weeks 4, 24, and 40. The primary analysis for arterial stiffness will be as per the primary outcome. A regression model with arterial stiffness at 40 weeks as the outcome variable, and treatment group and baseline arterial stiffness included as covariates in the model will be fitted. A secondary analysis will also be conducted using a longitudinal repeated measures analysis without the random statement. The variables treatment group (with chlortalidone as the reference group) and baseline arterial stiffness will be included in the model as a covariates. The repeated statement variable will be time. Analyses will be conducted in Stata using the xtreg command or SAS using the proc mixed command.

Safety Evaluation
The proportion of patients reporting hyperkalemia, a decline in renal function (requiring discontinuation of trial therapy), symptomatic hypotension (requiring discontinuation of trial therapy) and proportion of patients reporting side-effects (requiring discontinuation of trial therapy) will be analyzed as categorical variables using a chi-squared test, with relative risks and 95% confidence intervals reported. The proportion of patients who died and the
proportion of patients who experience a cardiovascular event will be compared in the two
treatment groups. A relative risk and 95% confidence interval will be reported, but no
hypothesis testing will be performed.

Impact
For most subjects with early stage CKD, the risk of death greatly exceeds the risk of
progression to end stage renal disease and much of the premature mortality is due to
cardiovascular disease. There is a pressing need therefore, for research in to
treatments to reduce the cardiovascular mortality and morbidity of this high risk group.
Published randomized trials of treatments to reduce cardiovascular risk in subjects with early
stage CKD are few. In the SHARP trial, statins reduced atherosclerotic events but had a
disappointing impact on total cardiovascular mortality suggesting that much cardiovascular
disease in CKD is not atherosclerotic. This result is in line with epidemiological data
suggesting that in CKD, sudden death, heart failure and stroke are much more common than
myocardial infarction. While ACE inhibitors are commonly used in subjects with CKD and
may have risk reducing effects similar to those seen in the general population, physicians
have tended to avoid MRB drugs because of concerns about hyperkalemia.
In a previous study we showed that LV mass and arterial stiffness were improved with
spironolactone with few hyperkalemic problems in subjects with stages 2 and 3 CKD under
close monitoring. This trial will further examine the use of spironolactone in a similar cohort
and will specifically test the hypothesis that the effects of spironolactone on LV mass
demonstrated in the CRIB-2 study were due to specific MRB mediated effects and not to
non-specific blood pressure lowering effects. This hypothesis has biological plausibility in
view of the known cardiovascular inflammatory, fibrotic and hypertrophic actions of
aldosterone and the antagonistic effects of MRB drugs on these processes.
Furthermore, in the 4E study of subjects with LVH, the addition of eplerenone to enalapril
causd significant reduction in LV mass with little effect on blood pressure. (31) Cardiac MRI
is the method of choice for determining changes in LV mass in response to treatment due to
its high inter-study reproducibility, lack of dependence on geometric assumptions and independence of changes in volume.\(^{(32, 33)}\) Spironolactone may be a powerful treatment to reverse or delay the onset of LVH and myocardial fibrosis in patients with CKD. As both hypertrophy and fibrosis are likely key intermediate phenotypic changes that result in an increased risk of heart failure and arrhythmia in many patient groups, the data will be of great value in establishing a mode of action of these drugs that may be relevant to subjects with CKD and other at risk groups.\(^{(34)}\) The range of secondary end points including arterial stiffness, aortic distensibility, LV fibrosis, systolic and diastolic function, kidney function and biomarkers may also provide powerful evidence of differences in the effects of MRB and diuretic drugs.

The effect of thiazide like drugs on LV mass in early stage CKD has not previously been examined although they are effective in lowering blood pressure in this group. The study will also provide evidence on the safety of both spironolactone and chlorthalidone in subjects with early stage CKD giving valuable data on the effects of both drugs on kidney function measured by eGFR and on changes in serum potassium and sodium concentrations. If safety and efficacy are confirmed, the rationale would be provided for a larger clinical trial designed to examine the effects of MRB drugs on cardiovascular morbidity and mortality end points. It is acknowledged that the non-blinded study design increases the risk of bias but the PROBE design maintains the benefits associated with a strict randomization procedure while the blinded endpoints help to eliminate bias. In a meta-analysis of trials of an angiotensin receptor blocker, changes in mean 24-h ambulatory blood pressure in double blind and PROBE trials were not significantly different.\(^{(35)}\)

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4. References


