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Effect of spironolactone on cardiovascular morbidity and mortality in patients with hypertension and glucose metabolism disorders (ESCAM): a study protocol for a pragmatic randomised controlled trial

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ABSTRACT

Introduction Hypertension combined with diabetes and hypokalemia is more likely to develop hyperaldosteronism and is at higher risk of cardiovascular events. There is evidence that activation of aldosterone and mineralocorticoid receptors may play a significant role in the occurrence of cardiovascular events in patients with hypertension and diabetes. Clinical studies have demonstrated that spironolactone can reduce the incidence of cardiovascular events in patients with chronic kidney diseases or severe heart failure. However, the effect of spironolactone on cardiovascular risk in patients with hypertension and glucose metabolism disorders (GMD) and low potassium has been scarcely studied. Therefore, this study aims to evaluate whether add-on spironolactone (conventional antihypertensive drugs alone vs conventional antihypertensive drugs+spironolactone) can reduce the morbidity and mortality of cardiovascular events in this population.

Methods and analysis In this multicentre, randomised, parallel-controlled study, a total of 7140 hypertensive patients aged 45–75 years with GMD and low potassium will be randomised in a 1:1 manner to the control or the spironolactone group (20 mg/day or with a maximum dose of 40 mg). The primary objective is to estimate the difference in the HR of composite cardiovascular events between the two groups. We will also assess the effects of spironolactone on individual cardiovascular events and the progression of diabetes and renal dysfunction.

Ethics and dissemination This protocol was approved by the Independent Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region (no. 2020020618). The results will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration number ChiCTR2000028909.

Strengths and limitations of this study

- This randomised controlled trial will be the first large-sample study to directly evaluate the cardiovascular efficacy of spironolactone in patients with hypertension and glucose metabolism disorders.
- This trial has primary end points of cardiovascular events.
- This trial will address a problem with an intervention design, which will be pragmatic and will have high potential application value in real-world setting.
- This study is designed as an open-label trial, and participants and investigators are not blinded to the treatment.

INTRODUCTION

Multiple comorbidities have been associated with hypertension. Studies have found that the prevalence of primary aldosteronism (PA) among patients diagnosed with hypertension is 10%[1 2] and reaches more than 15% in patients with resistant hypertension[3 4]; however, current evidence considers that the prevalence of PA may be underestimated.[5 6] In addition to elevating blood pressure (BP), aldosterone also causes aggravation of insulin resistance and diabetes by lowering the level of potassium.[7 8] Hypertensive patients with PA are more likely to be associated with impaired glucose tolerance and diabetes.[9 10] Therefore, it is reasonable to speculate that patients with hypertension are more prone to hyperaldosteronism when glucose metabolism disorders (GMD) and lower potassium exist simultaneously, and aldosterone may play an important role in the high incidence of cardiovascular events in this specific population. As well known, hypertension and GMD are common comorbidities,[11] and clinicians tend to pay more attention to the known pathogenesis, whereas they ignore the role of aldosterone. Meanwhile, aldosterone-induced

hypokalemia is often falsely attributed to the use of diuretics.

The risk of cardiovascular events is higher when hypertension and GMD coexist than when one of them is alone.[12] The two conditions, concurrently occurring in the majority of patients, show the negative effects of agents in lowering BP, aggravate target organ damages and increase the incidence of cardiovascular events.[13 14] In this process, hypertension and GMD share several common known pathogenesis, including the overactivation of the sympathetic nervous system, the activation of the renin-angiotensin-aldosterone system and insulin resistance.[15] Accordingly, correlative antihypertensive medicines are used against these mechanisms. However, the difficult-to-control BP and disproportionate increase in cardiovascular events in this population suggest that the coexistence of hypertension and GMD may be far more complicated than the simple combination of the two conditions.[16] As evidenced, patients with hypertension and diabetes usually need two or more antihypertensive agents to achieve the target BP as well.

Previous studies have shown that plasma aldosterone concentrations are higher in patients with both hypertension and diabetes than in patients with hypertension alone.[17 18] It has been also observed that the expression and sensibility of mineralocorticoid receptors (MR) are increased in patients with diabetes .[19 20] Furthermore, aldosterone breakthrough, related to endothelial dysfunction, left ventricular function deterioration and renal damage, occurs in about 50% of patients treated with ACE inhibitors or angiotensin receptor blockers, which are the preferred agents for patients with hypertension and GMD.[21–23] The MR-dependent mechanisms of aldosterone are also related to the pathophysiology of hypertension and cardiovascular diseases.[24 25] Evidence from human and animal studies suggests that aldosterone and MR activation play important roles in increasing the risk of cardiovascular events by promoting endothelial dysfunction, inflammation, vascular oxidative stress and fibrosis and by the imbalance of vasomotor factors.[26–28] Aldosterone also impairs arterial compliance, induces cardiac hypertrophy and increases left ventricular mass.[29]

Taken together, it is reasonable to believe that spironolactone, the aldosterone antagonist, can be more effective in patients with hypertension and GDM, and add-on spironolactone would provide a cardiovascular benefit to this population. In several small-sample clinical trials, MR antagonists (MRAs) significantly reduce BP and urinary protein in patients with hypertension and diabetes mellitus.[20 30 31] Increasing clinical studies have shown that MRAs reduce the incidence of cardiovascular events and all-cause mortality in patients with chronic kidney disease or heart failure.[32–35] However, the antihypertensive efficacy and safety of MRAs in patients with hypertension and GDM have not been demonstrated in large clinical trials, and it is unclear whether the MRAs bring an extra cardiovascular benefit for patients with hypertension and GDM and low potassium.

Therefore, this pragmatic clinical trial is designed to evaluate whether add-on spironolactone is more effective than conventional antihypertensive drugs in reducing incident cardiovascular events in patients with hypertension and GDM and low potassium.

METHODS

Overview of study design

The Effect of Spironolactone on CArdiovascular Morbidity and Mortality in patients with hypertension and glucose metabolism disorders (ESCAM) study is a multicentre, parallel-group, pragmatic, randomised controlled trial to be conducted in China. The primary objective is to test the hypothesis that add-on spironolactone is more effective than conventional antihypertensive drugs alone in reducing the incidence of cardiovascular events in patients with hypertension and GDM and low potassium. The study design is shown in figure 1. The schedule for enrolment, interventions and assessments is presented

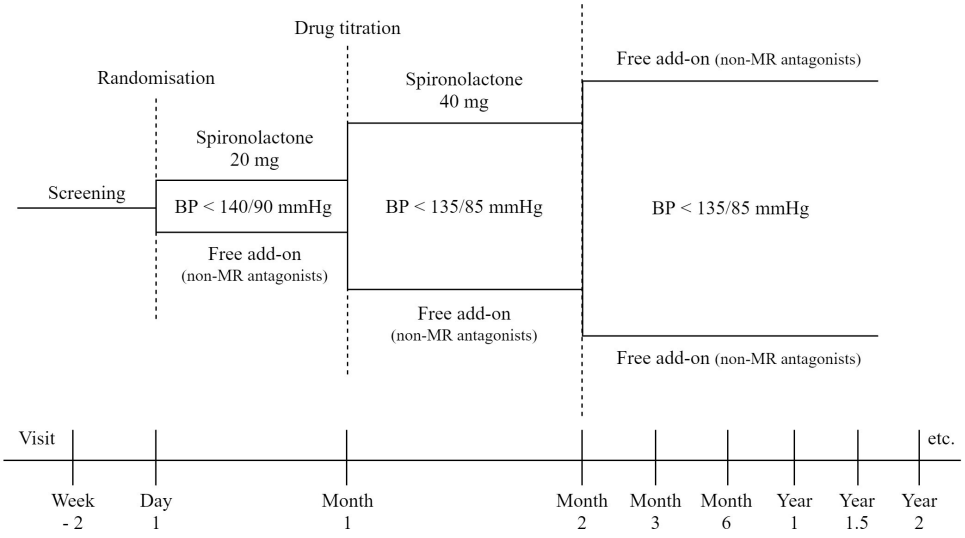

Figure 1 Study design for the ESCAM study. BP, blood pressure; MR, mineralocorticoid receptors.

Table 1 Study schedule for patients

	Study period									
	Screening	Baseline	Follow-up (months)							
Time point	0	0	1	3	6	12	18	24	30	36
Enrolment										
Eligibility screen	X									
Informed consent	X									
Randomisation		X								
Interventions										
Conventional antihypertensive drugs		X	X	X	X	X	X	X	X	X
Conventional antihypertensive drugs+spironolactone		X	X	X	X	X	X	X	X	X
Assessments										
Demographics		X								
Medical history		X								
Records of treatments		X	X	X	X	X	X	X	X	X
Blood pressure		X	X	X	X	X	X	X	X	X
Blood test		X		X	X	X		X		X
Glycometabolism test		X		X	X	X		X		X
Kidney function test		X		X	X	X		X		X
ECG		X			X	X		X		X
Cardiovascular events			X	X	X	X	X	X	X	X
Adverse events			X	X	X	X	X	X	X	X
Concomitant treatment		X	X	X	X	X	X	X	X	X
Adherence			X	X	X	X	X	X	X	X

ECG, electrocardiogram.

in table 1. A checklist in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) is also available in online supplemental table S1.

Eligible participants will be randomised to the spironolactone treatment group or the control group using an allocation ratio of 1:1 after a 2-week screening period. Other necessary treatments are allowed at clinicians' suggestions. Three committees will be established to supervise the whole process of the trial. The Executive Committee will provide guidance and make decisions about the design, execution and publication. The Data Safety and Monitoring Board will review and evaluate the safety and efficacy data of the trial. The Study Protocol Management Committee will address specific challenges in protocol procedures. Study reporting adheres to SPIRIT Reporting.[36]

Study objectives

Primary objective

The primary objective is to evaluate whether add-on spironolactone is more effective than conventional antihypertensive medicines alone in reducing the incidence of composite cardiovascular events in patients with hypertension and GDM and low potassium.

- Composite cardiovascular events include death due to cardiovascular causes, heart failure, myocardial infarction (MI), stroke, unstable angina admission, coronary revascularisation and atrial fibrillation.

Secondary objective

The secondary objective is to evaluate whether add-on spironolactone is more effective than usual antihypertensive medicines alone in reducing the incidence of the following outcomes:

- All-cause death.
- MI (fatal and non-fatal).
- Stroke (fatal and non-fatal).
- Heart failure.
- Atrial fibrillation.

Other objectives

To compare the effects on BP, glucose metabolism and renal function (serum creatinine, estimated glomerular filtration rate, blood urea nitrogen, uric acid, urine

Box 1 Inclusion criteria for the ESCAM study

Inclusion criteria

- Male or female patients aged 45–75 years and able to provide informed consent.
- Office-seated systolic blood pressure (BP) ≥140 mm Hg and/or diastolic BP ≥90 mm Hg, and/or under antihypertension treatment.
- Fasting blood glucose ≥6.1 mmol/L or postprandial glucose ≥7.8 mmol/L, or diagnosed diabetes.
- Plasma potassium <4.0 mmol/L.

protein and the incidence of dialysis) between the two treatment groups.

Study population

Participant recruitment is expected to be completed by 31 December 2020. The means by which participants are recruited are as follows: (1) recruitment advertisement via internet and media, (2) recommended by clinicians, (3) recommended by participants and (4) screening of the hospital electronic medical system and health examination data. Male and female patients aged 45–75 years diagnosed with hypertension and GMD as well as low serum potassium will be included in the study. Hypertension is defined as office systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg, and/or under antihypertension treatment. GMD includes impaired fasting glucose, impaired glucose tolerance and diagnosed diabetes. The details of the inclusion and exclusion criteria are shown in boxes 1 and 2.

Sample size

The ESCAM study is designed to detect a 20% reduction in the primary end point for the add-on spironolactone group, compared with the control group, with 80% power at 5% significance level during a 3-year follow-up. Assuming a cardiovascular event rate of 10% in the control group in a mean follow-up duration of 3 years and a lost-to-follow-up rate of 10%, a sample size of 7140 is required. The sample size was calculated using the PASS software V.11.0, and the parameters are as follows: α=0.05, β=0.2, P1 (treatment group)=8%, P2 (control group)=10% and alternative=two-sided.

Box 2 Exclusion criteria for the ESCAM study

Exclusion criteria

- History of cardiovascular events within the last 3 months (including MI, heart failure, stroke, unstable angina, coronary revascularisation and coronary bypass operation).
- Renal dysfunction (Scr ≥178 µmol/L or eGFR <60 mL/min).
- Hepatic dysfunction (AST/ALT >5 ULN or ALP >5 ULN or BIL >3 ULN).
- Serum uric acid >520 µmol/L.
- Diagnosed with secondary or resistant hypertension (including Cushing syndrome, adrenal tumour, pheochromocytoma, renal hypertension, polycystic ovary syndrome and congenital adrenal disease).
- Diagnosed with primary aldosteronism and is under spironolactone therapy.
- Diagnosed with malignant tumour within the last 5 years.
- Pregnant or breastfeeding women.
- Contraindicated or allergic to spironolactone.
- Severe mental disorders.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIL, bilirubin; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; ULN, upper limit of normal.

Randomisation

Randomisation of treatment allocation will be accomplished by a phone call to the study centre. Stratified by sex, block randomisation will be used, with random block sizes of 4. Eligible participants will be given a sequential random number based on a list generated by R statistical software and will be assigned to the spironolactone or the control group. The random sequence will be kept by the study centre, and the allocation concealment will be preserved for the participants and study investigators.

Intervention

After the screening visit, participants will enter a prerandomisation phase that lasts up to 2 weeks, and qualified patients will then enter the treatment period. Participants already receiving antihypertensive therapy will remain on their previous regimen. For the spironolactone group, patients will be given an add-on spironolactone of 20 mg, allowing titration to a maximum dose of 40 mg according to the target BP (<135/85 mm Hg) during follow-up. For the control group, patients will be given antihypertensive treatment according to clinicians' suggestions to reach a target BP <135/85 mm Hg, whereas the use of MRAs is limited. A blood glucose target will be set for the treatment of GMD (target fasting blood glucose <8.0 mmol/L, postprandial blood glucose <10 mmol/L), but the hypoglycaemic agents are not specialised. Other appropriate medications for concomitant conditions will be allowed for all participants throughout the study.

Follow-up and data acquisition

All qualified participants will be given a similar schedule of visits. After visit 1 (day 1), participants visit the study centres at month 1, month 2, month 3 and month 6 and thereafter every 6 months until the occurrence of the end points or the end of the study. Follow-up schedule will be planned to end at 31 December 2023. Predesigned structural questionnaires and case report forms (CRF) will be applied to collect all required data, including demographic data, laboratory examination, cardiovascular events, the use of medications and adverse events.

Safety and withdrawal

Safety and tolerability will be assessed by monitoring the occurrence of serious signs and adverse events during visits. Spironolactone will be used to reduce dosage or will to be discontinued when serum potassium rises above 5.0 mmol/L. Serious adverse events, defined as events that are fatal, life-threatening, disabling or will result in malformation, will be recorded in the CRF. Subjects may

withdraw from the trial at any time at their own request or at the decision of the Executive Committee for safe, behavioural or administrative reasons.

Management of the study

The overall responsibility for this trial is vested in the Executive Committee, which will provide guidance and make decisions about the design, execution and publication. The Data and Safety Monitoring Committee will be responsible for monitoring the safety of participants in this trial and for monitoring the relative efficacies of the two groups in terms of the number of cardiovascular events. This committee may recommend that the trial be discontinued prematurely when a sharp therapeutic advantage occurs in one of the treatment groups or when serious adverse events occur. The Study Protocol Management Committee will responsible for specific issues during the study.

Outcomes assessment

Cardiovascular death is defined as sudden death from cardiac cause or death due to heart failure, MI, stroke, cardiovascular invasive procedures, cardiovascular haemorrhage or other known vascular causes. MI needs to meet the criteria for ischaemic symptoms or corresponding electrocardiographic changes plus evidence of myocardial damage. Stroke includes haemorrhagic and ischaemic types, excluding subarachnoid haemorrhage. All-cause death includes death due to any reason. Evidence for death includes death certificates from hospitals or reports from family members.

In the ESCAM study, all outcomes will be identified according to the criteria we set in advance. Source data for all suspected cases will be submitted to the study centre for further verification, including medical records, imaging data and event report forms.

STATISTICAL ANALYSIS

Study data will be locked, and statistical analysis will be performed only with the permission of the principal investigator. The statistician will be blinded for treatment allocation. Any information related to participant identity will be erased before analyses. The primary analysis will be intention-to-treat. The cumulative event rates will be calculated using the Kaplan-Meier method and compared using the log-rank test. The HR and 95% CI will be estimated by the Cox proportional hazards regression model. The secondary end points will be analysed with the same methods used in primary analysis. Other efficacy and safety parameters will be summarised and compared for the differences between the two groups. Subgroup analyses will be performed, including gender, age (<60 years and ≥60 years), the classification of GMD, the absence or the presence of previous cardiovascular events, or the absence or the presence of baseline renal dysfunction. Statistical analyses will be performed using SPSS V.22.0 (SPSS Inc., Chicago, Illinois, USA).

ETHICS AND DISSEMINATION

This protocol was approved by the Independent Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region (no. 2020020618). Written informed consent to participate will be obtained from all participants. Patients' family will be allowed to do this when the patient is unable to provide written informed consent. Results will be disseminated in peer-reviewed journals and at scientific conferences.

PATIENT AND PUBLIC INVOLVEMENT STATEMENT

Participants are not directly involved in the design or development of the study and will not be involved in the recruitment and conduct of the study. Results of the therapeutic efficacy will be given to the participants after the study.

DISCUSSION

The morbidity and mortality of cardiovascular diseases remain high in patients with both hypertension and GMD, although several studies have reported that the combination of conventional antihypertensive medicines could reduce the risk of cardiovascular events.[37–39] Therefore, it is necessary to identify the potential risk factors and take interventions to further reduce the incidence of cardiovascular events. This trial will address the problem through a simple intervention, which will be pragmatic and will have high potential application value in real-world setting.

Previous studies have shown that the increase in aldosterone and the activation of MR may be closely associated with the occurrence and development of cardiovascular events.[26–28 40–44] A research on diabetic animals has demonstrated that treatment with spironolactone improves the deterioration of BP and blood glucose, and reduces the production of inflammatory factors.[30] Clinical studies have shown that low-dose spironolactone can effectively reduce BP in patients with diabetes and resistant hypertension[20 45] and also significantly reduce the incidence of cardiovascular events in patients with severe heart failure.[29] MRAs can not only reduce BP by directly blocking the MR[46] but may also bring the beneficial effects on cardiovascular events and mortality through the reduction of cardiovascular remodelling and vascular inflammation,[47 48] the alleviation of vascular calcification and aortic stiffness,[49] and the effects on oxidative stress and endothelial functions.[50] Therefore, it is reasonable to speculate that spironolactone treatment on top of conventional antihypertensive therapy would add further protection against cardiovascular events other than simply provide additional BP-lowering effect.

In the ESCAM study, spironolactone will be the only mandatory intervention drug, and patients' serum potassium will be closely monitored to ensure their safety. Both groups will have equal opportunities to have logical regimens to treat hypertension, GMD, dyslipidaemia

and other disorders, thereby avoiding the inequalities in these reactions, thus causing problems in interpreting outcomes in clinical trials. Moreover, this simple method of intervention is more pragmatic in real-world clinical therapy.

At present, the incidence of cardiovascular events in patients with hypertension and GDM is still high and even increasing in many developing countries. Evidence has shown that aldosterone and MR activation may play a significant role in cardiovascular events. However, it remains unknown whether MRA reduces the risk in this population. Therefore, this large pragmatic clinical trial is established to check the hypothesis that add-on spironolactone would significantly decrease the incidence of cardiovascular events. We expect that the results of the study would provide convincing evidence regarding the treatment in patients with both hypertension and GMD.

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Contributors NL conceived and designed the study, and led the proposal and protocol development. ML, QL, MH, LW, XY, JH and QZ participated in study design and planned the analyses. ML drafted the initial manuscript, and MH, YL and JY revised it carefully. All authors read and approved the final manuscript.

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REFERENCES

1 Fardella CE, Mosso L, Gómez-Sánchez C, *et al*. Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *J Clin Endocrinol Metab* 2000;85:1863–7.
2 Loh KC, Koay ES, Khaw MC, *et al*. Prevalence of primary aldosteronism among Asian hypertensive patients in Singapore. *J Clin Endocrinol Metab* 2000;85:2854–9.
3 Douma S, Petidis K, Doumas M, *et al*. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet* 2008;371:1921–6.
4 Florczak E, Prejbisz A, Szwench-Pietrasz E, *et al*. Clinical characteristics of patients with resistant hypertension: the RESIST-POL study. *J Hum Hypertens* 2013;27:678–85.
5 Byrd JB, Turcu AF, Auchus RJ. Primary aldosteronism. *Circulation* 2018;138:823–35.
6 Monticone S, Burrello J, Tizzani D, *et al*. Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol* 2017;69:1811–20.
7 Chatterjee R, Yeh H-C, Shafi T, *et al*. Serum and dietary potassium and risk of incident type 2 diabetes mellitus: the Atherosclerosis risk in communities (ARIC) study. *Arch Intern Med* 2010;170:1745–51.
8 Watanabe D, Yatabe M, Ichihara A. Evaluation of insulin sensitivity and secretion in primary aldosteronism. *Clin Exp Hypertens* 2016;38:613–7.
9 Monticone S, D'Ascenzo F, Moretti C, *et al*. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2018;6:41–50.
10 Akehi Y, Yanase T, Motonaga R, *et al*. High prevalence of diabetes in patients with primary aldosteronism (PA) associated with subclinical hypercortisolism and prediabetes more prevalent in bilateral than unilateral PA: a large, multicenter cohort study in Japan. *Diabetes Care* 2019;42:938–45.
11 Long AN, Dagogo-Jack S. Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. *J Clin Hypertens* 2011;13:244–51.
12 Zhao D, Liu J, Wang M, *et al*. Epidemiology of cardiovascular disease in China: current features and implications. *Nat Rev Cardiol* 2019;16:203–12.
13 Tian J, Sheng C-S, Sun W, *et al*. Effects of high blood pressure on cardiovascular disease events among Chinese adults with different glucose metabolism. *Diabetes Care* 2018;41:1895–900.
14 Liu H-H, Cao Y-X, Li S, *et al*. Impacts of prediabetes mellitus alone or plus hypertension on the coronary severity and cardiovascular outcomes. *Hypertension* 2018;71:1039–46.
15 Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. *Can J Cardiol* 2018;34:575–84.
16 American Diabetes Association. Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. American diabetes association. *Diabetes Care* 1989;12:573–9.
17 Karashima S, Yoneda T, Kometani M, *et al*. Angiotensin II receptor blocker combined with eplerenone or hydrochlorothiazide for hypertensive patients with diabetes mellitus. *Clin Exp Hypertens* 2016;38:565–70.
18 Li N, Wang M, Wang H, *et al*. Prevalence of primary aldosteronism in hypertensive subjects with hyperglycemia. *Clin Exp Hypertens* 2013;35:175–82.
19 Shibata H, Itoh H. Mineralocorticoid receptor-associated hypertension and its organ damage: clinical relevance for resistant hypertension. *Am J Hypertens* 2012;25:514–23.
20 Oxlund CS, Henriksen JE, Tarnow L, *et al*. Low dose spironolactone reduces blood pressure in patients with resistant hypertension and type 2 diabetes mellitus: a double blind randomized clinical trial. *J Hypertens* 2013;31:2094–102.
21 Sato A, Fukuda S. Effect of aldosterone breakthrough on albuminuria during treatment with a direct renin inhibitor and combined effect with a mineralocorticoid receptor antagonist. *Hypertens Res* 2013;36:879–84.
22 Schrier RW. Aldosterone 'escape' vs 'breakthrough'. *Nat Rev Nephrol* 2010;6:61.
23 Yoneda T, Takeda Y, Usukura M, *et al*. Aldosterone breakthrough during angiotensin II receptor blockade in hypertensive patients with diabetes mellitus. *Am J Hypertens* 2007;20:1329–33.
24 Shibata S, Mu S, Kawarazaki H, *et al*. Rac1 GTPase in rodent kidneys is essential for salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. *J Clin Invest* 2011;121:3233–43.
25 Catena C, Colussi G, Marzano L, *et al*. Aldosterone and the heart: from basic research to clinical evidence. *Horm Metab Res* 2012;44:181–7.
26 Ohmine T, Miwa Y, Takahashi-Yanaga F, *et al*. The involvement of aldosterone in cyclic stretch-mediated activation of NADPH oxidase in vascular smooth muscle cells. *Hypertens Res* 2009;32:690–9.
27 Bender SB, McGraw AP, Jaffe IZ, *et al*. Mineralocorticoid receptor-mediated vascular insulin resistance: an early contributor to diabetes-related vascular disease? *Diabetes* 2013;62:313–9.

28 Xiao F, Puddefoot JR, Vinson GP. Aldosterone mediates angiotensin II-stimulated rat vascular smooth muscle cell proliferation. *J Endocrinol* 2000;165:533–6.
29 Pitt B, Zannad F, Remme WJ, *et al*. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. randomized Aldactone evaluation study Investigators. *N Engl J Med* 1999;341:709–17.
30 Swaminathan K, Davies J, George J, *et al*. Spironolactone for poorly controlled hypertension in type 2 diabetes: conflicting effects on blood pressure, endothelial function, glycaemic control and hormonal profiles. *Diabetologia* 2008;51:762–8.
31 Saklayen MG, Gyebi LK, Tasosa J, *et al*. Effects of additive therapy with spironolactone on proteinuria in diabetic patients already on ACE inhibitor or ARB therapy: results of a randomized, placebo-controlled, double-blind, crossover trial. *J Investig Med* 2008;56:714–9.
32 Zannad F, McMurray JJV, Krum H, *et al*. Eplerenone in patients with systolic heart failure and mild symptoms. *N Engl J Med* 2011;364:11–21.
33 Vukusich A, Kunstmann S, Varela C, *et al*. A randomized, double-blind, placebo-controlled trial of spironolactone on carotid intima-media thickness in nondiabetic hemodialysis patients. *Clin J Am Soc Nephrol* 2010;5:1380–7.
34 Pitt B, Kober L, Ponikowski P, *et al*. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: a randomized, double-blind trial. *Eur Heart J* 2013;34:2453–63.
35 Chung Y-W, Yang Y-H, Wu C-K, *et al*. Spironolactone is associated with reduced risk of new-onset atrial fibrillation in patients receiving renal replacement therapy. *Int J Cardiol* 2016;202:962–6.
36 Chan A-W, Tetzlaff JM, Gøtzsche PC, *et al*. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
37 Lindholm LH, Ibsen H, Dahlöf B, *et al*. Cardiovascular morbidity and mortality in patients with diabetes in the losartan intervention for endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002;359:1004–10.
38 Jamerson K, Weber MA, Bakris GL, *et al*. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008;359:2417–28.
39 Weber MA, Bakris GL, Jamerson K, *et al*. Cardiovascular events during differing hypertension therapies in patients with diabetes. *J Am Coll Cardiol* 2010;56:77–85.
40 Sabbadin C, Calò LA, Armanini D. The story of spironolactones from 1957 to now: from sodium balance to inflammation. *G Ital Nefrol* 2016;33 Suppl 66:33.s66.12.
41 Kim SK, McCurley AT, DuPont JJ, *et al*. Smooth muscle cell-mineralocorticoid receptor as a mediator of cardiovascular stiffness with aging. *Hypertension* 2018;71:609–21.
42 Young MJ. Mechanisms of mineralocorticoid receptor-mediated cardiac fibrosis and vascular inflammation. *Curr Opin Nephrol Hypertens* 2008;17:174–80.
43 Thum T, Schmitter K, Fleissner F, *et al*. Impairment of endothelial progenitor cell function and vascularization capacity by aldosterone in mice and humans. *Eur Heart J* 2011;32:1275–86.
44 Cole TJ, Young MJ. 30 years of the mineralocorticoid receptor: mineralocorticoid receptor null mice: informing cell-type-specific roles. *J Endocrinol* 2017;234:T83–92.
45 Djoumessi RN, Noubiap JJN, Kaze FF, *et al*. Effect of low-dose spironolactone on resistant hypertension in type 2 diabetes mellitus: a randomized controlled trial in a sub-Saharan African population. *BMC Res Notes* 2016;9:187.
46 Lin C, Zhang Q, Zhang H, *et al*. Long-term effects of low-dose spironolactone on chronic dialysis patients: a randomized placebo-controlled study. *J Clin Hypertens* 2016;18:121–8.
47 Iraqi W, Rossignol P, Angioi M, *et al*. Extracellular cardiac matrix biomarkers in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure: insights from the eplerenone post-acute myocardial infarction heart failure efficacy and survival study (EPHESUS) study. *Circulation* 2009;119:2471–9.
48 Edwards NC, Steeds RP, Stewart PM, *et al*. Effect of spironolactone on left ventricular mass and aortic stiffness in early-stage chronic kidney disease: a randomized controlled trial. *J Am Coll Cardiol* 2009;54:505–12.
49 Voelkl J, Alesutan I, Leibrock CB, *et al*. Spironolactone ameliorates PIT1-dependent vascular osteoinduction in klotho-hypomorphic mice. *J Clin Invest* 2013;123:812–22.
50 Brown NJ. Contribution of aldosterone to cardiovascular and renal inflammation and fibrosis. *Nat Rev Nephrol* 2013;9:459–69.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	NA
Funding	4	Sources and types of financial, material, and other support	24
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 24
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	15
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4, 5 ,6
	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7

Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	11, 12
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13, 14
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13, 14
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13, 14
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10, 14-16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9(table 1) and figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14, 15
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16, 17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16,17
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16, 17

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	15
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7-9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	NA
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.