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Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis: study protocol for a pilot randomized controlled trial

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Abstract

Introduction: Endothelial and cardiac dysfunction are highly prevalent and are associated with cardiovascular morbidity and mortality among dialysis patients. Coenzyme Q10 (CoQ10) supplementation can improve endothelial dysfunction and left ventricular ejection fraction and reduce cardiovascular events in the general population. For dialysis patients, no study has explored the effect of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function. However, the issue remains uncertain. The aim of this study is to explore whether CoQ10 supplementation can improve endothelial and cardiac function in hemodialysis patients.

Methods and analysis: This is a pilot randomized controlled study. Eligible patients undergoing hemodialysis in our hemodialysis center will be randomly allocated to the CoQ10 and control group. The follow-up time is 12 months. The primary outcome is to assess the change of brachial artery flow-mediated dilation (FMD) at 12 months from baseline. The major secondary outcomes include: change in peak early mitral annulus velocity (e'), E/e' [ie, the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e')], grade of diastolic dysfunction, left ventricular ejection fraction, myocardial performance index, and left ventricular mass index at 12 months compared with baseline. Additional secondary outcomes are the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine), brain-natriuretic peptide level, CoQ10 concentration

from baseline, death or hospitalization due to cardiovascular events, and all-cause mortality.

Ethics and dissemination

Risks associated with coenzyme Q10 are minor. The trial has received ethics approval from the local ethics boards. The results of the study are expected to be published in a peer-reviewed journal and presented at academic conferences. If promising, data from the pilot study will be used for a larger randomized clinical trial.

Trial registration number: ChiCTR1900022258

Strengths and limitations of this study

The pilot randomized trial protocol was developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.

We will first evaluate the effect of coenzyme Q10 on endothelial function in patients undergoing hemodialysis.

Due to the pilot nature of this study with a small sample size, this study may not be able to result in significant therapeutic effects.

Introduction

Cardiovascular events are prevalent and the leading cause of death for patients undergoing hemodialysis patients [1] [2]. Endothelial cells play an important role in maintaining cardiovascular homeostasis and the development of cardiovascular pathologies [3]. Flow-mediated dilatation (FMD) test been established as a valid method for noninvasive assessment of endothelial function[4]. The systolic dysfunction, diastolic dysfunction, and left ventricular hypertrophy are frequent occurrences in dialysis patients, which are related to higher cardiovascular morbidity and mortality among dialysis patients [5-9]. Echocardiography is commonly used and can provide accurate information on left ventricular function, chamber dimension, and geometry, presence of left ventricular hypertrophy[5].

Coenzyme Q10 (CoQ10), as an important in vivo antioxidant, is an essential component of the mitochondrial electron transport chain [10]. Supplementation with CoQ10 may be of benefit to the general population. Previous studies have demonstrated that oral CoQ10 supplementation improved endothelial dysfunction in the general population [11] [12]. Meanwhile, treatment with CoQ10 can result in improvement in the left ventricular ejection fraction and reducing cardiovascular events and mortality [13-15].

For patients undergoing hemodialysis, no study has explored the effects of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function [16] [17]. However, the issue remains uncertain.

Thus, we will conduct this pilot randomized controlled study to evaluate the efficacy and safety of CoQ10 in hemodialysis patients and explore some parameters for a future clinical trial with a large sample size.

The trial protocol was written according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Statement [18]. A SPIRIT checklist is provided in Additional File 1.

Methods and Analysis

Ethics

This study will be performed in accordance with the principles of the Declaration of Helsinki. All patients must provide written informed consent before undergoing any study-related procedures. The study protocol has been approved by the Ethics Committee of the 306th Hospital of Chinese PLA. If any significant changes must be made to the protocol, a draft of the new version will be submitted for approval.

Study design

This pilot study is a parallel, single-center, randomized controlled trial. The study will be carried out in the hemodialysis center of the 306th Hospital of Chinese PLA. The study will be sequentially conducted as follows: enrollment according to prespecified inclusion and exclusion criteria, randomization, follow-up for 12 months, and assessment. Figure 1 demonstrates the flow chart of the study.

Participants and eligibility

All patients in our hemodialysis center will be eligible for screening according to the

inclusion and exclusion criteria.

Inclusion criteria

The inclusion criteria are as follows: undergo thrice-weekly hemodialysis for at least 3 months; aged more than 18 and less than 85 years; life expectancy greater than 1 year.

Exclusion criteria

Patients will be excluded if they have any of the following: poor adherence of dialysis or medications; severe systemic or local infection; malignancy; planning to receive kidney transplant within 12 months; hospitalization within 30 days; history of a major atherosclerotic event within 3 months; pregnancy or lactation; current use antioxidant other than vitamin C; use of hemodialysis catheter.

Randomization procedure, allocation concealment, and intervention

An independent biometrician with no relationship to the data management and data statistical analysis will use the Stata software (version 16.0) to generate random numbers. The allocation ratio is 1:1. The allocation codes will be placed in sealed opaque envelopes until participants are randomized. The participants and echocardiographer will be blind to group allocation. If there is a serious adverse reaction, an emergency unblinding procedure will be initiated. Participants will be randomized to CoQ10 or placebo group. The CoQ10 group will receive oral CoQ10 (Puritan's Pride, USA) 400 mg once daily. Both CoQ10 and placebo will be orally administered for 12 months. Patients enrolled in the trial will continue to receive and undergo all usual clinical care activities.

Participants can withdraw from the trial for any reason at any time. Participants will be withdrawn from the study if they violate any of the key inclusion or exclusion criteria or they refuse to continue to participate or withdraw their consent, or the investigators judge that they need to be withdrawn from the study.

Sample size

Because of the lack of adequate preliminary studies in hemodialysis patients and the pilot nature of this study, we have adopted 30 participants in each group [19 20]. Posters to encourage patients to enroll in the clinical trial will be posted in our hemodialysis center.

Study outcomes

Primary outcome

The primary outcome is the change in brachial artery FMD from baseline after 12 months of treatment. The endothelial function will be assessed by brachial artery FMD with ultrasound equipment on the arm free of vascular access [21-23]. FMD will be assessed by an observer who is blinded to treatment allocation and will be tested at baseline and every 6 months. All vasodilation medication will have to be interrupted for at least 4 h before the examination, if possible. Patients were instructed to avoid heavy meals, caffeine or smoking 12 hours prior to the FMD measurements. The measurements will be in a temperature-controlled room and will start after 15 min of rest in the supine position. A baseline image of the brachial artery will be obtained above the antecubital fossa in a longitudinal plane. Then a sphygmomanometer will be inflated for 5 min, at least 50 mmHg above the systolic pressure, and no more than

300 mmHg. The brachial diameter was measured at 60 seconds following cuff deflation at the same position. The FMD is defined as at the 60-second time-point as ([post-inflation diameter) − (baseline diameter])/baseline diameter) × 100%.

Major secondary outcomes

Prespecified major secondary outcomes are echocardiographic outcomes change in peak early mitral annulus velocity (e'), E/e' [ie, the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e')], grade of diastolic dysfunction, left ventricular ejection fraction, myocardial performance index, and left ventricular mass index (LVMI) at 12 months compared with baseline.

Evaluation of left ventricular diastolic function will be according to updated recommendations from the European Association of Cardiovascular Imaging (EACVI) and American Society of Echocardiography (ASE) for the evaluation of diastolic function by echocardiography published in 2016 [24]. Peak early mitral inflow velocity (E) and peak late mitral inflow velocity (A) will be measured from the tip of the mitral leaflets of the left ventricular apical four-chamber view. Peak tricuspid regurgitation (TR) systolic jet velocity will be obtained during systole at the leading edge of the spectral waveform from the four-chamber view, with the angle-adjusted alignment of continuous wave Doppler echo beam. Tissue Doppler imaging technique will be used to determine e' (both septal and lateral mitral annular areas were evaluated). E/e' and E/A were also calculated. Left atrial volumes were determined using two-dimensional (2D) echocardiography. Indexed LAV (LAVI) will be calculated by dividing LAV by body surface area.

Left ventricular ejection fraction will be determined as: (LVEDV-LVEDSV)/LVEDV × 100, where LVEDV and LVESV represent the left ventricular end-diastolic volume and left ventricular end-systolic volume.

The myocardial performance index is a combined index of systolic and diastolic function and was calculated as (a − b)/b where interval (a) is equal to the sum of isovolumic contraction, isovolumic relaxation time and ejection time(from the cessation to the onset of mitral inflow) and interval (b) represents the ejection time (obtained at the ventricular outflow tract). Thus, the sum of isovolumic contraction and relaxation time was obtained by subtracting (b) from (a) [25 26].

Left ventricular mass (LVM) was calculated according to a previously published methodology. LVM (g) = 0.8{1.04× [(LVEDD + IVST + PWT)^3 – (LVEDD)^3] + 0.6, where LVEDD, IVST and PWT are left ventricular end-diastole diameter, interventricular septum thickness, and posterior wall thickness at end-diastole, respectively. LVMI (g/m^2) was calculated as follows: LVMI = Left ventricular mass / body surface area [27].

All echocardiographic examinations will be performed by an observer who is blinded to clinical data.

Additional secondary outcomes

Additional secondary outcomes are the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine), brain-natriuretic peptide level, CoQ10 concentration from baseline, death or hospitalization due to cardiovascular events, and all-cause mortality.

Adverse events

All adverse events related to CoQ10 will be reported to the Ethics Committee of the 306th Hospital of Chinese PLA in written case report form. Safety will be monitored using routine blood examination, liver function, blood electrolytes and so on.

Follow-up protocol

Patients will be followed up clinically every 3 months until the end of the study at 12 months. Biochemical data will be collected at baseline and every 3 months including hemoglobin, urea, creatinine, albumin, calcium, phosphate, intact parathyroid hormone, brain natriuretic peptide, and high-sensitivity C-reactive protein measured by standard methods. Kt/V values will be also calculated and collected. FMD test and echocardiographic examinations will be performed at baseline, 6 and 12 months after discharge. Participant adherence to the protocol will be monitored by interviews at study check-up visits to promote participant retention and complete follow-up. In order to assess medication adherence, the participants will be asked to take the study medication that is left over for weighing at each clinical visit.

Data collection, management, and monitoring

The data collected at baseline and follow-up visits will fill in the Case Report Forms. Original medical records and informed consents are archived in the participating centers and saved for at least 5 years after the clinical trials finish. All data will be transferred to the data statistical units for data entry and management with the EpiData3.1 database. The data will be entered independently into the database by two researchers and will be checked separately by different trained researchers. The

privacy of the participants is guaranteed. Each participant will receive a participation identity number in this study, with which personal information of participants is labeled in papers. Personal information will be kept in a locked storage unit by one researcher who has will have access to the final trial dataset.

An independent data and safety monitoring board will oversee all aspects of the study. The board will meet periodically during the trial to monitor safety. It will make recommendations on study progress and performance, identify any major adverse outcomes or adverse outcomes due to the therapy, and give advice regarding whether the study should continue or if there should be a protocol change.

Harms

For hemodialysis patients, daily CoQ10 supplementation at doses as high as 1800 mg was safe and well-tolerated [17] [28]. Potential adverse events include gastrointestinal discomfort, loss of appetite, nausea, diarrhea, and rash. In the process of the clinical trial, any severe adverse events must be immediately reported to the Ethics Committee of the 306th Hospital of Chinese PLA within 24 h and recorded including the time of occurrence, severity, duration, measures, and outcome.

Statistical methods

The intent-to-treat (ITT) analysis set will be used as the principal analysis for efficacy analyses. All patients who have begun treatment will be included irrespective of their protocol adherence and continued participation in the study. Patients who complete the study and comply well with the study protocol without major protocol violations will constitute the per-protocol set. The per-protocol analysis will be used as the

secondary analysis for efficacy analyses. Missing data will be handled using the last observation carried forward method. Analysis of covariance (ANCOVA) was used to analyze the change from baseline in primary and secondary outcomes adjusted for baseline values and treatment assignment. A 2-tailed $P<0.05$ will be used as the cutoff for statistical significance. All statistical analyses will be performed using Stata software (version 16.0).

Patient and public involvement

The study participants were not involved beyond the standard roles as the subjects of the proposed trial. The public was not involved.

Dissemination

We plan to report the trial results for publication in an appropriate journal and to communicate the results at an academic conference. Our final report will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines [29].

Discussion

CoQ10 is a key component in energy transduction and the antioxidant process [30]. CoQ10 supplementation can be used for the treatment of endothelial dysfunction. For patients with ischemic heart disease, a 1-month treatment of CoQ10 significantly improved FMD from 4.6±0.6 to 7.8± 0.6%, whereas there was no change in the control group [12]. Hamilton et al. found brachial artery FMD was improved by 1% after CoQ10 supplementation for 12 weeks in type 2 diabetic patients [31]. A meta-analysis combined five eligible randomized controlled trials showed that treatment with CoQ10 significantly improved in endothelial function assessed

peripherally by flow-mediated dilatation (standard mean difference 1.70, 95% CI: 1.00-2.4, p<0.0001) [11].

The detailed mechanism might involve altering local vascular oxidative stress [12 31]. Tiano's study indicated that improvements of extracellular superoxide dismutase (ecSOD) activity might be related to CoQ10 capability of enhancing endothelial function [12]. In this study, patients with lower levels of ecSOD had greater improvement in endothelial function.

Excessive oxidative stress is highly prevalent and correlated with cardiovascular morbidity and mortality for hemodialysis patients [32]. The excessive oxidative stress might result from due to loss of antioxidants during dialysis and activation of white blood cells triggering the production of reactive oxygen species [33]. Although no study has investigated the effect of CoQ10 supplementation on endothelial function in dialysis patients, increasing evidence has indicated that CoQ10 supplementation can effectively decrease oxidative stress in this special population [17 34-36].

Regarding cardiac function, for the general population, a meta-analysis showed treatment with CoQ10 had a favorable effect on left ventricular ejection fraction [15]. One randomized controlled trial (Q-SYMBIO trial) [14] and one recent meta-analysis[13] found CoQ10 treatment significantly reduced major adverse cardiovascular events and mortality.

For hemodialysis patients, to our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function or biomarker of cardiac function[16 17]. One randomized controlled trial has explored the

efficacy of CoQ10 supplementation on cardiac function and found that CoQ10 supplementation decreased left ventricular mass and left ventricular posterior wall as well as interventricular septum thickness, and did not improve diastolic heart function in this special population [16]. However, this study, being a cross-over trial, only had a sample size with a total of 28 participants and short follow-up time with only 8 weeks in each phase and 4-week washout period. Another small sample study [17] indicated that no significant effect of CoQ10 treatment on N-terminal pro-B-type natriuretic peptide (NT-proBNP) was found. However, in the per-protocol analysis, significantly lower levels of NT-proBNP among patients assigned to 1200 mg CoQ10 compared to placebo were found. To date, no study has focused on the effects of CoQ10 treatment on cardiovascular events and mortality.

Based on existing evidence, we hypothesize that the administration of CoQ10 will have a favorable effect on endothelial dysfunction and cardiac function in hemodialysis patients.

However, this is a pilot trial with a small sample size. Hence, this study may not be able to result in significant therapeutic effects. The present trial is a study of great value for it will provide important parameters for a future trial with a large sample size.

Abbreviations

CoQ10: Coenzyme Q10; A: peak late mitral inflow velocity; ANCOVA: analysis of covariance; E: peak early mitral inflow velocity; e': peak early mitral annulus velocity; E/e': the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e'); ecSOD: extracellular superoxide dismutase; FMD: flow-mediated dilation; ITT: Intention-to-treat analysis; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVM: Left ventricular mass; LVMI: left ventricular mass index; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

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Financial and other competing interests

The authors declare no financial and other competing interests.

Availability of data and materials

The data collected will be made available through requests sent to the lead author.

Contributors

Yongxing Xu and Jianjun Gao conceived the trial idea. Yongxing Xu, Jianjun Gao, Xinlou Li, and Xiaowen Zuo drafted the trial protocol and all others contributed to the final version. Yongxing Xu and Jianjun Gao generated the original draft of the manuscript and all other authors contributed. Xinlou Li, Xiaowen Zuo, and Huaping Jia provided expert advice in the design of the study. All authors approved the final version for submission.

Figure 1. Study flow chart.

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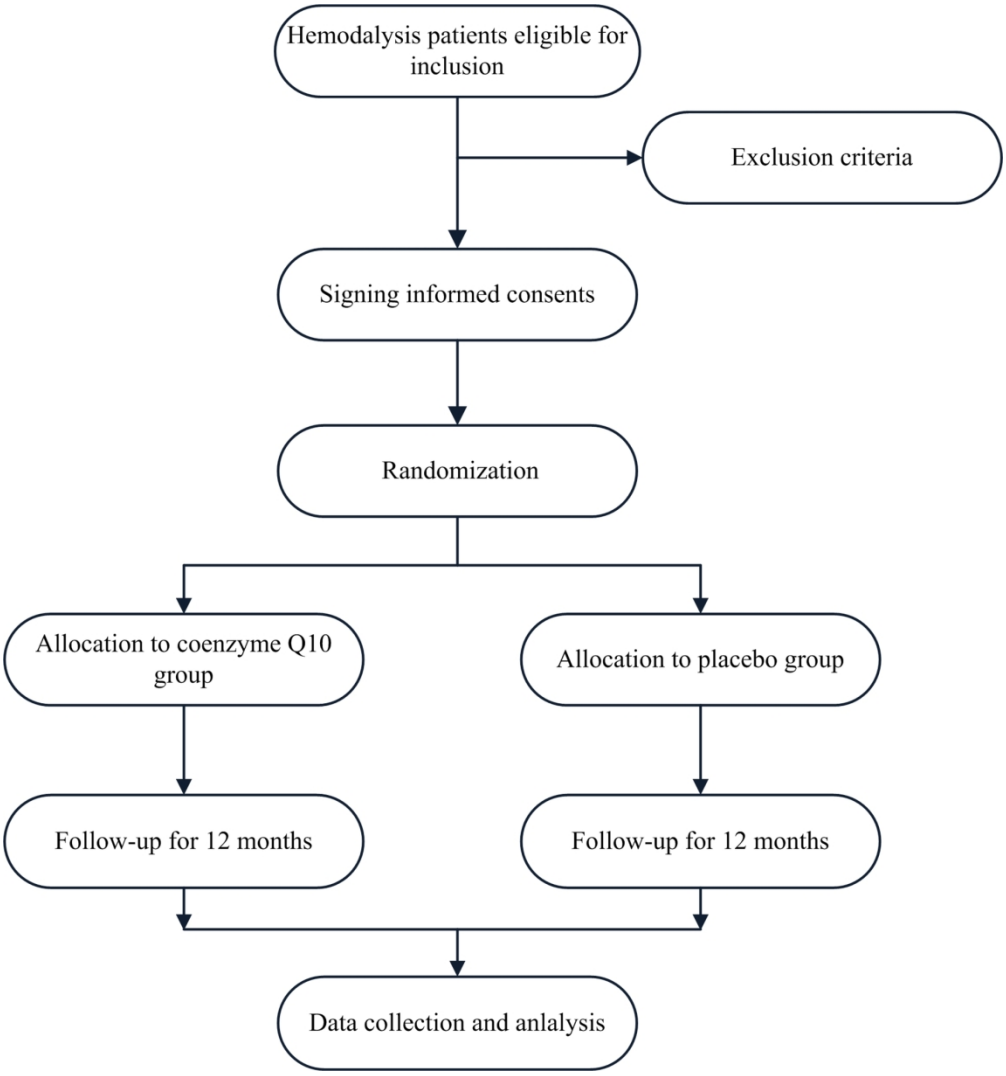

Figure 1. Study flow chart.

164x175mm (300 x 300 DPI)

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

Section/item	Item No	Description	Page Number on which item is reported
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	Not applicable
Protocol version	3	Date and version identifier	14
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,15
	5b	Name and contact information for the trial sponsor	15
	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	15
	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)	10-11
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

	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
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, noninferiority, exploratory)	5,6
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	5
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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	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)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
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	7-10
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)	5,10

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	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	6
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	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
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	10

	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	10
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	10
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	11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	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)	11-12
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	11
	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	Not applicable
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	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5

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)	5
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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	11
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	Not applicable
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	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not applicable
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	Not applicable

*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.

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Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis: study protocol for a pilot randomized controlled trial

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Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis: study protocol for a pilot randomized controlled trial

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Word count: 2758

Keywords: coenzyme Q10, endothelial function, cardiac function, hemodialysis, protocol.

Abstract

Introduction: Endothelial and cardiac dysfunction are highly prevalent and are associated with cardiovascular morbidity and mortality among dialysis patients. For dialysis patients, no study has explored the effect of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function. However, the effect of CoQ10 supplementation on cardiac function remains uncertain in hemodialysis patients. The aim of this study is to explore whether CoQ10 supplementation can improve endothelial and cardiac function in hemodialysis patients.

Methods and analysis: This is a pilot randomized controlled study. Eligible patients undergoing hemodialysis in our hemodialysis center will be randomly allocated to the CoQ10 and control group. The follow-up time is 12 months. The primary outcome is to assess the change of brachial artery flow-mediated dilation (FMD) at 12 months from baseline. The major secondary outcomes include: change in peak early mitral annulus velocity (e'), E/e' [ie, the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e')], grade of diastolic dysfunction, left ventricular ejection fraction, myocardial performance index, and left ventricular mass index at 12 months compared with baseline. Additional secondary outcomes are the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine), brain-natriuretic peptide level, CoQ10 concentration from baseline, death or hospitalization due to cardiovascular events, and all-cause

mortality.

Ethics and dissemination

Risks associated with coenzyme Q10 are minor, even at doses as high as 1800 mg according to previous studies. The trial has received ethics approval from the Medical Ethics Committee for Clinical Trials of Drugs, the 306th Hospital of Chinese PLA. The results of the study are expected to be published in a peer-reviewed journal and presented at academic conferences.

Trial registration number: ChiCTR1900022258

Strengths and limitations of this study

A major strength of the proposed study is that we will first evaluate the effect of coenzyme Q10 on endothelial function in patients undergoing hemodialysis.

We will use hard endpoints including cardiovascular events and all-cause mortality as prespecified outcomes.

A limitation of the study is that the sample size is relatively small, which can result in insufficient power to determine effects.

Introduction

Cardiovascular events are prevalent and the leading cause of death for patients undergoing hemodialysis patients [1] [2]. Endothelial cells play an important role in maintaining cardiovascular homeostasis and the development of cardiovascular pathologies [3]. Flow-mediated dilatation (FMD) test been established as a valid method for noninvasive assessment of endothelial function[4]. The systolic dysfunction, diastolic dysfunction, and left ventricular hypertrophy are frequent occurrences in dialysis patients, which are related to higher cardiovascular morbidity and mortality among dialysis patients [5-9]. Echocardiography is commonly used and can provide accurate information on left ventricular function, chamber dimension, and geometry, presence of left ventricular hypertrophy[5].

Coenzyme Q10 (CoQ10), as an important in vivo antioxidant, is an essential component of the mitochondrial electron transport chain [10]. Supplementation with CoQ10 may be of benefit to the general population. Previous studies have demonstrated that oral CoQ10 supplementation improved endothelial dysfunction in the general population [11] [12]. Meanwhile, treatment with CoQ10 can result in improvement in the left ventricular ejection fraction and reducing cardiovascular events and mortality [13-15].

For patients undergoing hemodialysis, no study has explored the effects of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function [16] [17]. However, the effect of CoQ10 supplementation on cardiac

function remains uncertain in hemodialysis patients.

Thus, we will conduct this pilot randomized controlled study to evaluate the efficacy and safety of CoQ10 in hemodialysis patients and explore some parameters for a future clinical trial with a large sample size.

The trial protocol was written according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Statement [18]. A SPIRIT checklist is provided in Additional File 1.

Methods and Analysis

Study design

This pilot study is a parallel, single-center, randomized controlled trial. The study will be carried out in the hemodialysis center of the 306th Hospital of Chinese PLA. The study will be sequentially conducted as follows: enrollment according to prespecified inclusion and exclusion criteria, randomization, and follow-up for 12 months, and assessment. Figure 1 demonstrates the flow chart of the study.

Participants and eligibility

All patients in our hemodialysis center will be eligible for screening according to the inclusion and exclusion criteria.

Inclusion criteria

The inclusion criteria are as follows: undergoing thrice-weekly hemodialysis for at least 3 months, aged more than 18 and less than 85 years, life expectancy greater than 1 year.

Exclusion criteria

Patients will be excluded if they have any of the following: poor adherence of dialysis or medications; severe systemic or local infection; malignancy; planning to receive kidney transplant within 12 months; hospitalization within 30 days; history of a major atherosclerotic event within 3 months; pregnancy or lactation; current use antioxidant other than vitamin C; use of hemodialysis catheter.

Randomization procedure, allocation concealment, and intervention

An independent biometrician with no relationship to the data management and data statistical analysis will use the Stata software (version 16.0) to generate random numbers. The allocation ratio is 1:1. The allocation codes will be placed in sealed opaque envelopes until participants are randomized. The participants and echocardiographer will be blind to group allocation. If there is a serious adverse reaction, an emergency unblinding procedure will be initiated. Participants will be randomized to CoQ10 or placebo group. The CoQ10 group will receive oral CoQ10 (Puritan's Pride, USA) 400 mg once daily. Both CoQ10 and placebo will be orally administered for 12 months. Patients enrolled in the trial will continue to receive and undergo all usual clinical care activities.

Participants can withdraw from the trial for any reason at any time. Participants will be withdrawn from the study if they violate any of the key inclusion or exclusion criteria or they refuse to continue to participate or withdraw their consent, or the investigators judge that they need to be withdrawn from the study.

Sample size

Because of the lack of adequate preliminary studies in hemodialysis patients and the

pilot nature of this study, we have adopted 30 participants in each group [19 20]. Posters to encourage patients to enroll in the clinical trial will be posted in our hemodialysis center.

Study outcomes

Primary outcome

The primary outcome is the change in brachial artery FMD from baseline after 12 months of treatment. The endothelial function will be assessed by brachial artery FMD with ultrasound equipment on the arm free of vascular access [21-23]. FMD will be assessed by an observer who is blinded to treatment allocation and will be tested at baseline and every 6 months. All vasodilation medication will have to be interrupted for at least 4 h before the examination, if possible. Patients were instructed to avoid heavy meals, caffeine or smoking 12 hours prior to the FMD measurements. The measurements will be in a temperature-controlled room and will start after 15 min of rest in the supine position. A baseline image of the brachial artery will be obtained above the antecubital fossa in a longitudinal plane. Then a sphygmomanometer will be inflated for 5 min, at least 50 mmHg above the systolic pressure, and no more than 300 mmHg. The brachial diameter was measured at 60 seconds following cuff deflation at the same position. The FMD is defined as at the 60-second time-point as ([post-inflation diameter) − (baseline diameter])/baseline diameter) × 100%.

Major secondary outcomes

Prespecified major secondary outcomes are echocardiographic outcomes change in peak early mitral annulus velocity (e'), E/e' [ie, the ratio between peak early mitral

inflow velocity (E) and peak early mitral annulus velocity (e')], grade of diastolic dysfunction, left ventricular ejection fraction, myocardial performance index, and left ventricular mass index (LVMI) at 12 months compared with baseline.

Evaluation of left ventricular diastolic function will be according to updated recommendations from the European Association of Cardiovascular Imaging (EACVI) and American Society of Echocardiography (ASE) for the evaluation of diastolic function by echocardiography published in 2016 [24]. Peak early mitral inflow velocity (E) and peak late mitral inflow velocity (A) will be measured from the tip of the mitral leaflets of the left ventricular apical four-chamber view. Peak tricuspid regurgitation (TR) systolic jet velocity will be obtained during systole at the leading edge of the spectral waveform from the four-chamber view, with the angle-adjusted alignment of continuous wave Doppler echo beam. Tissue Doppler imaging technique will be used to determine e' (both septal and lateral mitral annular areas were evaluated). E/e' and E/A were also calculated. Left atrial volumes were determined using two-dimensional (2D) echocardiography. Indexed LAV (LAVI) will be calculated by dividing LAV by body surface area.

Left ventricular ejection fraction will be determined as: (LVEDV-LVEDSV)/LVEDV × 100, where LVEDV and LVESV represent the left ventricular end-diastolic volume and left ventricular end-systolic volume.

The myocardial performance index is a combined index of systolic and diastolic function and was calculated as (a − b)/b where interval (a) is equal to the sum of isovolumic contraction, isovolumic relaxation time and ejection time(from the

cessation to the onset of mitral inflow) and interval (b) represents the ejection time (obtained at the ventricular outflow tract). Thus, the sum of isovolumic contraction and relaxation time was obtained by subtracting (b) from (a) [25 26].

Left ventricular mass (LVM) was calculated according to a previously published methodology. LVM (g) = 0.8{1.04× [(LVEDD + IVST + PWT)^3 – (LVEDD)^3] + 0.6, where LVEDD, IVST and PWT are left ventricular end-diastole diameter, interventricular septum thickness, and posterior wall thickness at end-diastole, respectively. LVMI (g/m^2) was calculated as follows: LVMI = Left ventricular mass / body surface area [27].

All echocardiographic examinations will be performed by an observer who is blinded to treatment allocation.

Additional secondary outcomes

Additional secondary outcomes are the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine), brain-natriuretic peptide level, CoQ10 concentration from baseline, death or hospitalization due to cardiovascular events, and all-cause mortality.

Adverse events

All adverse events related to CoQ10 will be reported to the ethics committee in written case report form. Safety will be monitored using routine blood examination, liver function, blood electrolytes and so on.

Follow-up protocol

Patients will be followed up clinically every 3 months until the end of the study at 12

months. Biochemical data will be collected at baseline and every 3 months including hemoglobin, urea, creatinine, albumin, calcium, phosphate, intact parathyroid hormone, brain natriuretic peptide, and high-sensitivity C-reactive protein measured by standard methods. Kt/V values will be also calculated and collected. The inferior vena cava collapsibility index was used to evlaluate volume status[28]. The inferior vena cava was measured by one observer who was blinded to treatment allocation in a supine position during expiration and maximal inspiration, avoiding valsalva-like manoeuvres. The inferior vena cava collapsibility index was calculated using the standard formula: (maximal diameter on expiration - minimal diameter on deep inspiration)/maximal diameter on expiration × 100%. The inferior vena cava collapsibility index, FMD test, and echocardiographic examinations will be performed at baseline, 6 and 12 months.

Participant adherence to the protocol will be monitored by interviews at study check-up visits to promote participant retention and complete follow-up. In order to assess medication adherence, the participants will be asked to take the study medication that is left over for weighing at each clinical visit.

Data collection, management, and monitoring

The data collected at baseline and follow-up visits will fill in the Case Report Forms. Original medical records and informed consents are archived in the participating centers and saved for at least 5 years after the clinical trials finish. All data will be transferred to the data statistical units for data entry and management with the EpiData3.1 database. The data will be entered independently into the database by two

researchers and will be checked separately by different trained researchers. The privacy of the participants is guaranteed. Each participant will receive a participation identity number in this study, with which personal information of participants is labeled in papers. Personal information will be kept in a locked storage unit by one researcher who has will have access to the final trial dataset.

An independent data and safety monitoring board will oversee all aspects of the study. The board will meet periodically during the trial to monitor safety. It will make recommendations on study progress and performance, identify any major adverse outcomes or adverse outcomes due to the therapy, and give advice regarding whether the study should continue or if there should be a protocol change.

Harms

For hemodialysis patients, daily CoQ10 supplementation at doses as high as 1800 mg was safe and well-tolerated [17] [29]. Potential adverse events include gastrointestinal discomfort, loss of appetite, nausea, diarrhea, and rash. In the process of the clinical trial, any severe adverse events must be immediately reported to the ethics committee within 24 h and recorded including the time of occurrence, severity, duration, measures, and outcome.

Statistical methods

The intent-to-treat (ITT) analysis set will be used as the principal analysis for efficacy analyses. All patients who have begun treatment will be included irrespective of their protocol adherence and continued participation in the study. Patients who complete the study and comply well with the study protocol without major protocol violations

will constitute the per-protocol set. The per-protocol analysis will be used as the secondary analysis for efficacy analyses. Missing data will be handled using the last observation carried forward method. Analysis of covariance (ANCOVA) was used to analyze the change from baseline in primary and secondary outcomes adjusted for baseline values and treatment assignment. A 2-tailed $P<0.05$ will be used as the cutoff for statistical significance. All statistical analyses will be performed using Stata software (version 16.0).

Patient and public involvement

The study participants were not involved beyond the standard roles as the subjects of the proposed trial. The public was not involved.

Ethics and dissemination

This study will be performed following the principles of the Declaration of Helsinki. All patients must provide written informed consent (Additional File 2) before undergoing any study-related procedures. The study protocol has been approved by the Medical Ethics Committee for Clinical Trials of Drugs, the 306th Hospital of Chinese PLA. If any significant changes must be made to the protocol, a draft of the new version will be submitted for approval.

We plan to report the trial results for publication in an appropriate journal and to communicate the results at an academic conference. Our final report will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines [30].

Discussion

CoQ10 is a key component in energy transduction and the antioxidant process [31].

CoQ10 supplementation can be used for the treatment of endothelial dysfunction. For patients with ischemic heart disease, a 1-month treatment of CoQ10 significantly improved FMD from 4.6±0.6 to 7.8± 0.6%, whereas there was no change in the control group [12]. Hamilton et al. found brachial artery FMD was improved by 1% after CoQ10 supplementation for 12 weeks in type 2 diabetic patients [32]. A meta-analysis combined five eligible randomized controlled trials showed that treatment with CoQ10 significantly improved in endothelial function assessed peripherally by flow-mediated dilatation (standard mean difference 1.70, 95% CI: 1.00-2.4, p<0.0001) [11].

The detailed mechanism might involve altering local vascular oxidative stress [12 32]. Tiano's study indicated that improvements of extracellular superoxide dismutase (ecSOD) activity might be related to CoQ10 capability of enhancing endothelial function [12]. In this study, patients with lower levels of ecSOD had greater improvement in endothelial function.

Excessive oxidative stress is highly prevalent and correlated with cardiovascular morbidity and mortality for hemodialysis patients [33]. The excessive oxidative stress might result from due to loss of antioxidants during dialysis and activation of white blood cells triggering the production of reactive oxygen species [34]. Although no study has investigated the effect of CoQ10 supplementation on endothelial function in dialysis patients, increasing evidence has indicated that CoQ10 supplementation can effectively decrease oxidative stress in this special population [17 35-37].

Regarding cardiac function, for the general population, a meta-analysis showed

treatment with CoQ10 had a favorable effect on left ventricular ejection fraction [15]. One randomized controlled trial (Q-SYMBIO trial) [14] and one recent meta-analysis[13] found CoQ10 treatment significantly reduced major adverse cardiovascular events and mortality.

For hemodialysis patients, to our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function or biomarker of cardiac function[16 17]. One randomized controlled trial has explored the efficacy of CoQ10 supplementation on cardiac function and found that CoQ10 supplementation decreased left ventricular mass and left ventricular posterior wall as well as interventricular septum thickness, and did not improve diastolic heart function in this special population [16]. However, this study, being a cross-over trial, only had a sample size with a total of 28 participants and short follow-up time with only 8 weeks in each phase and 4-week washout period. Another small sample study [17] indicated that no significant effect of CoQ10 treatment on N-terminal pro-B-type natriuretic peptide (NT-proBNP) was found. However, in the per-protocol analysis, significantly lower levels of NT-proBNP among patients assigned to 1200 mg CoQ10 compared to placebo were found. To date, no study has focused on the effects of CoQ10 treatment on cardiovascular events and mortality.

Based on existing evidence, we hypothesize that the administration of CoQ10 will have a favorable effect on endothelial dysfunction and cardiac function in hemodialysis patients. Compared with the existing studies, we will first evaluate the effect of CoQ10 treatment on endothelial dysfunction in hemodialysis patients. We

will use both echocardiographic parameters and biomarkers of cardiac function to evaluate the cardiac effect of CoQ10. Hard endpoints including cardiovascular events and mortality will be also prespecified outcomes in our study.

However, this is a pilot trial with a small sample size. Hence, this study may not be able to result in significant therapeutic effects. The present trial is a study of great value for it will provide important parameters, which can be used to evaluate the feasibility and safety of the protocol and calculate the sample size for a future trial with a larger sample size to determine whether coenzyme Q10 supplementation confers improved survival and reduced cardiovascular events in hemodialysis patients.

Abbreviations

CoQ10: Coenzyme Q10; A: peak late mitral inflow velocity; ANCOVA: analysis of covariance; E: peak early mitral inflow velocity; e': peak early mitral annulus velocity; E/e': the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e'); ecSOD: extracellular superoxide dismutase; FMD: flow-mediated dilation; ITT: Intention-to-treat analysis; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVM: Left ventricular mass; LVMI: left ventricular mass index; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

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Financial and other competing interests

The authors declare no financial and other competing interests.

Availability of data and materials

The data collected will be made available through requests sent to the lead author.

Contributors

Yongxing Xu and Jianjun Gao conceived the trial idea. Yongxing Xu, Jianjun Gao, Xinlou Li, and Xiaowen Zuo drafted the trial protocol and Enhong Han, Fugui Liang, and Lei Xie contributed to the final version. Yongxing Xu and Jianjun Gao generated the original draft of the manuscript and all authors contributed the final version. Xinlou Li, Xiaowen Zuo, and Huaping Jia provided expert advice in the design of the study. All authors approved the final version for submission.

Figure 1. Study flow chart.

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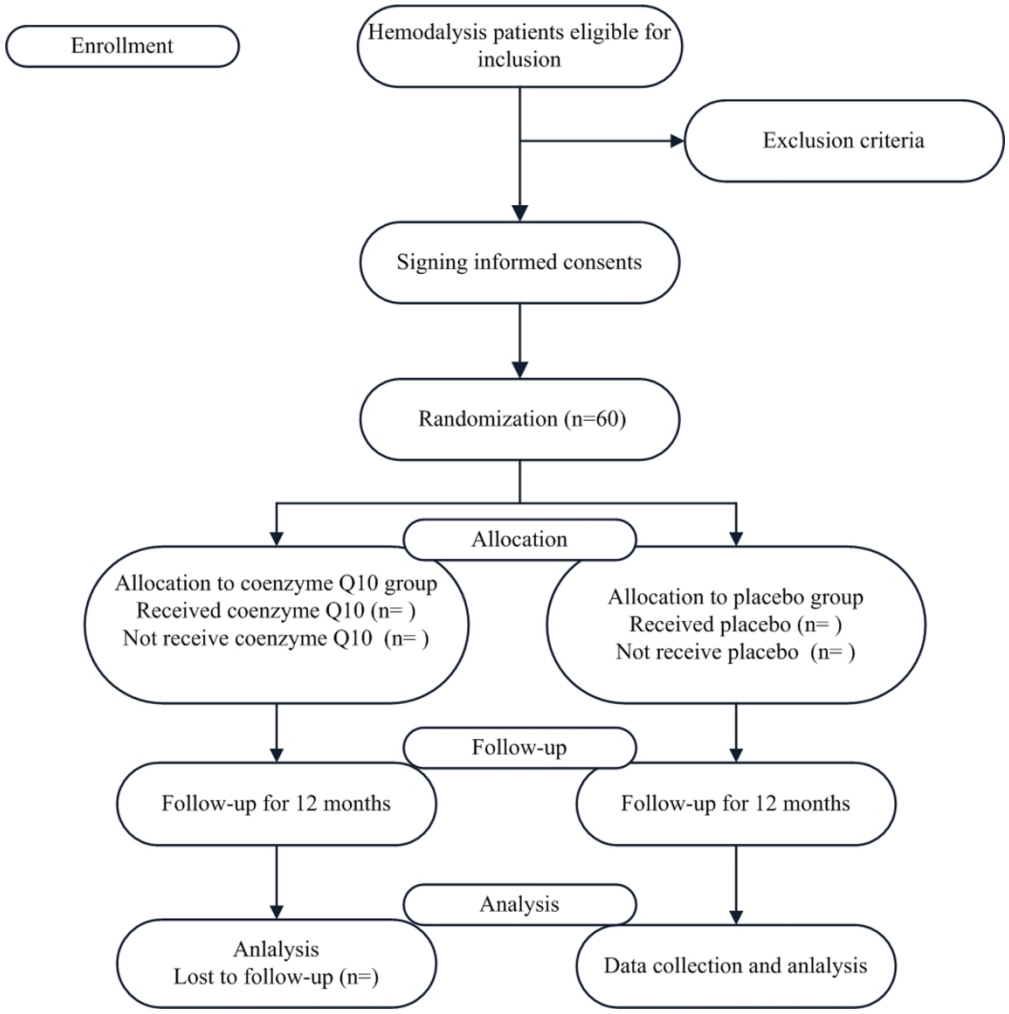

Figure 1. Study flow chart.

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	Page Number on which item is reported
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	Not applicable
Protocol version	3	Date and version identifier	16
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,17
	5b	Name and contact information for the trial sponsor	16
	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	16
	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)	10-11
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

	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
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, noninferiority, exploratory)	5,6
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	5
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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	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)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
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	7-9
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)	5,10

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	6-7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	6
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	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
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	10-11

	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	10-11
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	10-11
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	11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	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)	11-12
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	11
	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	Not applicable
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	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12

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)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
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	11
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	Not applicable
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	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional File 2
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	Not applicable

*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.

Consent form

（Version 03, November 5, 2018）

Name of subject：_____

Binding address：_____

Telephone number：_____

Dear Mr. / Madam_____:

We sincerely invite you to participate in the clinical trial of "Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis". This study is conducted in the hemodialysis center of the 306th Hospital of Chinese PLA. Before you agree to participate in this study, please read this consent form carefully. The consent form will provide you with the research background, purpose, method, benefits, the possible risk, and your rights and interests protection during the trial. The information provided in this consent form can help you decide whether to participate in this study or not. If you have any questions, please consult the researchers of the project to ensure that you fully understand the content. If you agree to participate in this study, please sign the consent form and keep a consent form signed by the two sides. This research program has been approved by the ethics committee of the 306th Hospital.

1. Why participate in this study?

Cardiovascular events are highly prevalent. Endothelial and cardiac dysfunction are frequent occurrences in dialysis patients, which are related to higher cardiovascular morbidity and mortality among dialysis patients. Excessive oxidative stress is highly prevalent and also correlated with cardiovascular morbidity and mortality for hemodialysis patients. The excessive oxidative stress might result from due to loss of antioxidants during dialysis and activation of white blood cells triggering the production of reactive oxygen species.

Coenzyme Q10 (CoQ10) is an important in vivo antioxidant. Supplementation with CoQ10 may be of benefit to the general population. Previous studies have demonstrated that oral CoQ10 supplementation improved endothelial dysfunction in the general population. Meanwhile, treatment with CoQ10 can result in improvement in the left ventricular ejection fraction, heart failure, and reducing cardiovascular events and mortality.

Increasing evidence has indicated that CoQ10 supplementation can effectively decrease oxidative stress in dialysis patients. One randomized controlled trial has explored the efficacy of CoQ10 supplementation on cardiac function and found that CoQ10 supplementation decreased left ventricular mass and left ventricular posterior wall as well as interventricular septum thickness, and did not improve diastolic heart function in this special population. Another small sample study indicated that no significant effect of CoQ10 treatment on N-terminal pro-B-type natriuretic peptide (NT-proBNP) was found. However, in the per-protocol analysis, significantly lower levels of NT-proBNP among patients assigned to 1200 mg CoQ10 compared to placebo were found.

Thus, we will conduct this pilot randomized controlled study to evaluate the efficacy and safety of CoQ10 in hemodialysis patients.

This study has been approved by the ethics committee of the 306th Hospital of Chinese PLA. This study complies with the relevant Chinese laws and regulations, the Helsinki declaration and other ethical principles to protect the rights and interests of subjects.

2. Who can take part in the study?

（1）Inclusion criteria

The inclusion criteria are as follows: undergo thrice-weekly hemodialysis for at least 3 months; aged more than 18 and less than 85 years; life expectancy greater than 1 year.

（2）Exclusion criteria

Patients will be excluded if they have any of the following: poor adherence of dialysis or medications; severe systemic or local infection; malignancy; planning to receive kidney transplant within 12 months; hospitalization within 30 days; history of a major atherosclerotic event within 3 months; pregnancy or lactation; current use antioxidant other than vitamin C; use of hemodialysis catheter.

3. How many people participated in the study?

The study is expected to recruit 60 eligible patients. The allocation ratio is 1:1. Participants will be randomized to CoQ10 or placebo group. Both the CoQ10 and placebo will be indistinguishable from each other in shape, size, color and packaging. You may be randomly assigned to the CoQ10 group or placebo group (the probability of being assigned to each group is 50%), but you can only enter only one group for treatment and evaluation. No matter which group you will be assigned, you will be given the appropriate basis and symptomatic treatment for your condition.

4. What is the research procedure?

We will first screen the hemodialysis patients, select the population with inclusion and exclusion criteria, and sign consent form with the patients. After agreeing to be the subject of our study, you will be followed up clinically every 3 months until the end of the study at 12 months. Biochemical data will be collected at baseline and every 3 months including hemoglobin, urea, creatinine, albumin, calcium, phosphate, intact parathyroid hormone, brain natriuretic peptide, and high-sensitivity C-reactive protein measured by standard methods. Kt/V values will be also calculated and collected. FMD test and echocardiographic examinations will be performed at baseline, 6 and 12 months. You will be followed up regularly for 12 months.

5. Possible risks and adverse reactions associated with participation in this study.

(1) The possible side effects and adverse reactions of study drugs

This study will provide you with CoQ10 or placebo. For hemodialysis patients, daily CoQ10 supplementation at doses as high as 1800 mg was safe and well-tolerated according

previous studies. Potential adverse events include gastrointestinal discomfort, loss of appetite, nausea, diarrhea, and rash. If you have any discomfort, new changes in your condition or any unexpected circumstances whether or not related to the drug, you should inform the research doctor in time, and the research doctor will make judgment and medical treatment.

(2) Blood drawing risks: including transient, mild pain, local bruises, mild dizziness in a few people, or extremely rare needle infections.

(3) Other risks: there may also be some other risks, discomfort, drug interactions or adverse reactions that are currently unknown.

6. What are the benefits of participating in this study?

One possible scenario is that you may not directly benefit from this study. Another possible scenario is that your endothelial function or cardiac function will be improved if you are allocated to treatment group if the effects of CoQ10 do exist. But we cannot guarantee this. Although participating in this study may not bring you immediate benefits, your participation may bring benefits to future dialysis patients.

7. If you don't participate in this study, is there any alternative treatment?

If you decide not to participate in this study, you will receive and undergo all usual clinical care activities including thrice-weekly dialysis, regularly monitor cliniacal and laboratory parameters and so on. Your researcher will suggest a treatment plan that suits you. Your researcher will also be happy to explain the possible benefits and risks of other treatments for your disease.

8. The cost of participating in the study

If you participate in this study, you can get free access to CoQ10 or placebo, free measurement for brachial artery flow-mediated dilation, and free echocardiography examination for three times. The above tests and inspection fees shall be borne by this study. However, the medication for the basic treatment and the examination or test items beyond the above instructions shall be at your expense.

9. Management of the occurrence of research-related injuries

If you suffer any adverse events related to this study or cause you any injury during the study period, the researcher will positively treat you and assume relevant responsibilities according to law. The sponsor (investigator) will not be responsible for your medical expenses if you do not comply with the requirements set forth by the investigator under this clinical trial protocol.

10. Voluntary participation and withdrawal from the test

You may choose not to participate in the study, or withdraw consent form from the study after being notified by the researchers at any time without discrimination or retaliation. Your medical treatment and rights will not be affected.

If you cannot make decision immediately, you have enough time to consider, if necessary, you can consult with relatives, friends and other people you trust before making decision.

The investigator may terminate your participation in the study if you require additional diagnosis/treatment, or if you do not follow the study plan, or for any other reasonable reason.

If you decide to withdraw early from a study, it is important that you consult the researchers about what other procedures to follow. During the study period, you may keep in touch with the information related to you in the study.

11. Your personal information will be strictly protected

If you decide to participate in the study, your personal information in and about the study will be kept confidential. Responsible for research physicians and other researchers will use your medical information for research. This information may include your name, address, telephone number, medical history, and information obtained during your study visit. To ensure that the research is conducted in accordance with the regulations, the sponsor, the pharmaceutical administration or the members of the ethics review committee are required to have access to your personal data at the research institute when necessary. Your personal information will not be disclosed when the results of this study are published.

12. Other items

1. For the sake of your health, the researcher may withdraw you from this study without your consent if:

If you continue to participate in this study, the risks may outweigh the benefits;

You do not participate in the study according to the study protocol instructed by the researcher;

The test is terminated prematurely.

2. We still recommend that you take necessary contraceptive measures during the trial if you or your partner during the trial become pregnant, tell the researcher or your physician immediately.

13. Who should you contact if you have any questions or difficulties?

If you have any questions about this study or if you have any discomfort or injury in the course of this study, please contact your research physician: _____。
Telephone:_____。

If you have any questions about your rights and interests during the research, please contact the biomedical ethics committee of our hospital at:_____。

Consent form·Signature page

Subject consent form statement

I have read this consent form and fully understand all the contents.

I have the opportunity to ask questions and all questions have been answered.

I understand that participation in this study is voluntary.

I can choose not to participate in this study, and my medical treatment and benefits will not be affected.

If I need additional treatment, or if I do not follow the study plan, or if there is a research-related injury or for any other reason, the study physician may terminate my continued participation in the study.

I will receive a signed copy of the informed consent.

Name of subject: _____ Signed by legal agent: _____
Signature of the subject: _____ Relationship with subjects: _____
Telephone of subject: _____ Legal representative telephone: _____

Date: Year Month Day Date: Year Month Day
(Note: legal representative's signature is required if subject is incapacitated)

Investigator notification statement

I have informed the subject or his/her legal representative of the purpose, methods, procedures, risks and benefits of the study in detail; Give him/her enough time to read the informed consent form, discuss with others, and answer all questions he/she raises; I have informed the subject of the contact information when encountering problems; I have informed the subject or his/her legal representative that he/she does not need any reason to withdraw from the study at any time during the study.

Name of the investigator: _____

The investigator's signature: _____

Researcher telephone: _____

Date: Year Month Day

BMJ Open

Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis: study protocol for a pilot randomized controlled trial

Journal:	*BMJ Open*
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Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis: study protocol for a pilot randomized controlled trial

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Abstract

Introduction: Endothelial and cardiac dysfunction are highly prevalent and are associated with cardiovascular morbidity and mortality among dialysis patients. For dialysis patients, no study has explored the effect of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function. However, the effect of CoQ10 supplementation on cardiac function remains uncertain in hemodialysis patients. The aim of this study is to explore whether CoQ10 supplementation can improve endothelial and cardiac function in hemodialysis patients.

Methods and analysis: This is a pilot randomized controlled study. Eligible patients undergoing hemodialysis in our hemodialysis center will be randomly allocated to the CoQ10 and control group. The follow-up time is 12 months. The primary outcome is to assess the change of brachial artery endothelial-dependent flow-mediated dilation (FMD), left ventricular systolic function, diastolic function, and myocardial performance index at 12 months from baseline. Secondary outcomes are death or hospitalization due to cardiovascular events, all-cause mortality, change of coenzyme Q10 concentration, the ratio of ubiquinol to ubiquinone, the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine), and left ventricular mass index.

Ethics and dissemination

Risks associated with coenzyme Q10 are minor, even at doses as high as 1800 mg

according to previous studies. The trial has received ethics approval from the Medical Ethics Committee for Clinical Trials of Drugs, the 306th Hospital of Chinese PLA. The results of the study are expected to be published in a peer-reviewed journal and presented at academic conferences.

Trial registration number: ChiCTR1900022258

Strengths and limitations of this study

A major strength of the proposed study is that we will first evaluate the effect of coenzyme Q10 on endothelial function in patients undergoing hemodialysis.

We will use hard endpoints including cardiovascular events and all-cause mortality as prespecified outcomes.

A limitation of the study is that the sample size is relatively small, which can result in insufficient power to determine effects.

Introduction

Cardiovascular events are prevalent and the leading cause of death for patients undergoing hemodialysis patients [1] [2]. Endothelial cells play an important role in maintaining cardiovascular homeostasis and the development of cardiovascular pathologies [3]. Flow-mediated dilatation (FMD) test been established as a valid method for noninvasive assessment of endothelial function[4]. The systolic dysfunction, diastolic dysfunction, and left ventricular hypertrophy are frequent occurrences in dialysis patients, which are related to higher cardiovascular morbidity and mortality among dialysis patients [5-9]. Echocardiography is commonly used and can provide accurate information on left ventricular function, chamber dimension, and geometry, presence of left ventricular hypertrophy[5].

Coenzyme Q10 (CoQ10), as an important in vivo antioxidant, is an essential component of the mitochondrial electron transport chain [10]. Supplementation with CoQ10 may be of benefit to the general population. Previous studies have demonstrated that oral CoQ10 supplementation improved endothelial dysfunction in the general population [11] [12]. Meanwhile, treatment with CoQ10 can result in improvement in the left ventricular ejection fraction and reducing cardiovascular events and mortality [13-15].

For patients undergoing hemodialysis, no study has explored the effects of supplementation of CoQ10 on endothelial function. To our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function [16] [17]. However, the effect of CoQ10 supplementation on cardiac

function remains uncertain in hemodialysis patients.

Thus, we will conduct this pilot randomized controlled study to evaluate the efficacy and safety of CoQ10 in hemodialysis patients and explore some parameters for a future clinical trial with a large sample size.

The trial protocol was written according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Statement [18]. A SPIRIT checklist is provided in Additional File 1.

Methods and Analysis

Study design

This pilot study is a parallel, single-center, randomized controlled trial. The study will be carried out in the hemodialysis center of the 306th Hospital of Chinese PLA. The study will be sequentially conducted as follows: enrollment according to prespecified inclusion and exclusion criteria, randomization, and follow-up for 12 months, and assessment. Figure 1 demonstrates the flow chart of the study.

Participants and eligibility

All patients in our hemodialysis center will be eligible for screening according to the inclusion and exclusion criteria.

Inclusion criteria

The inclusion criteria are as follows: undergoing thrice-weekly hemodialysis for at least 3 months, aged more than 18 and less than 85 years, life expectancy greater than 1 year.

Exclusion criteria

Patients will be excluded if they have any of the following: poor adherence of dialysis or medications; severe systemic or local infection; malignancy; planning to receive kidney transplant within 12 months; hospitalization within 30 days; history of a major atherosclerotic event within 3 months; pregnancy or lactation; current use antioxidant other than vitamin C; use of hemodialysis catheter.

Randomization procedure, allocation concealment, and intervention

An independent biometrician with no relationship to the data management and data statistical analysis will use the Stata software (version 16.0) to generate random numbers. The allocation ratio is 1:1. The allocation codes will be placed in sealed opaque envelopes until participants are randomized. The participants and echocardiographer will be blind to group allocation. If there is a serious adverse reaction, an emergency unblinding procedure will be initiated. Participants will be randomized to CoQ10 or placebo group. The CoQ10 group will receive oral CoQ10 (Puritan's Pride, USA) 400 mg once daily. Both CoQ10 and placebo will be orally administered for 12 months. Patients enrolled in the trial will continue to receive and undergo all usual clinical care activities.

Participants can withdraw from the trial for any reason at any time. Participants will be withdrawn from the study if they violate any of the key inclusion or exclusion criteria or they refuse to continue to participate or withdraw their consent, or the investigators judge that they need to be withdrawn from the study.

Sample size

Because of the lack of adequate preliminary studies in hemodialysis patients and the

pilot nature of this study, we have adopted 30 participants in each group [19 20]. Posters to encourage patients to enroll in the clinical trial will be posted in our hemodialysis center.

Study outcomes

Primary outcomes

The primary outcomes are the change in brachial artery endothelial-dependent FMD, left ventricular systolic function, diastolic function, and myocardial performance index from baseline after 12 months of treatment. The endothelial function will be assessed by brachial artery FMD with ultrasound equipment on the arm free of vascular access [21-23]. FMD will be assessed by an observer who is blinded to treatment allocation and will be tested at baseline and every 6 months. All vasodilation medication will have to be interrupted for at least 4 h before the examination, if possible. Patients were instructed to avoid heavy meals, caffeine or smoking 12 hours prior to the FMD measurements. The measurements will be in a temperature-controlled room and will start after 15 min of rest in the supine position. A baseline image of the brachial artery will be obtained above the antecubital fossa in a longitudinal plane. Then a sphygmomanometer will be inflated for 5 min, at least 50 mmHg above the systolic pressure, and no more than 300 mmHg. The brachial diameter was measured at 60 seconds following cuff deflation at the same position. The FMD is defined as at the 60-second time-point as ([post-inflation diameter) − (baseline diameter])/baseline diameter) × 100%.

Left ventricular systolic function will be assessed by left ventricular ejection fraction

determined as: (LVEDV-LVEDSV)/LVEDV × 100, where LVEDV and LVESV represent the left ventricular end-diastolic volume and left ventricular end-systolic volume.

Left ventricular diastolic function will be assessed by peak early mitral annulus velocity (e'), E/e' [ie, the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e')], grade of diastolic dysfunction. Evaluation of left ventricular diastolic function will be according to updated recommendations from the European Association of Cardiovascular Imaging (EACVI) and American Society of Echocardiography (ASE) for the evaluation of diastolic function by echocardiography published in 2016 [24]. Peak early mitral inflow velocity (E) and peak late mitral inflow velocity (A) will be measured from the tip of the mitral leaflets of the left ventricular apical four-chamber view. Peak tricuspid regurgitation (TR) systolic jet velocity will be obtained during systole at the leading edge of the spectral waveform from the four-chamber view, with the angle-adjusted alignment of continuous wave Doppler echo beam. Tissue Doppler imaging technique will be used to determine e' (both septal and lateral mitral annular areas were evaluated). E/e' and E/A were also calculated. Left atrial volumes were determined using two-dimensional (2D) echocardiography. Indexed LAV (LAVI) will be calculated by dividing LAV by body surface area.

The myocardial performance index is a combined index of systolic and diastolic function and was calculated as (a − b)/b where interval (a) is equal to the sum of isovolumic contraction, isovolumic relaxation time and ejection time(from the

cessation to the onset of mitral inflow) and interval (b) represents the ejection time (obtained at the ventricular outflow tract). Thus, the sum of isovolumic contraction and relaxation time was obtained by subtracting (b) from (a) [25 26].

All echocardiographic examinations will be performed by an observer who is blinded to treatment allocation.

Secondary outcomes

Secondary outcomes are death or hospitalization due to cardiovascular events, and all-cause mortality, the change of coenzyme Q10 concentration, the ratio of the reduced form of coenzyme Q10 (ubiquinol) to oxidized Coenzyme Q10 (ubiquinone), the change of oxidative stress markers (including malondialdehyde and 8-hydroxy-deoxyguanosine) and left ventricular mass index.

Left ventricular mass (LVM) was calculated according to a previously published methodology. LVM (g) = $0.8\{1.04\times [(\text{LVEDD} + \text{IVST} + \text{PWT})^3 - (\text{LVEDD})^3] + 0.6$, where LVEDD, IVST and PWT are left ventricular end-diastole diameter, interventricular septum thickness, and posterior wall thickness at end-diastole, respectively. LVMI (g/m^2) was calculated as follows: LVMI = Left ventricular mass/body surface area [27]. LVMI will be performed by an observer who is blinded to treatment allocation.

Adverse events

All adverse events related to CoQ10 and severe adverse events will be reported to the ethics committee in written case report form. Safety will be monitored using routine blood examination, liver function, blood electrolytes and so on.

Follow-up protocol

Patients will be followed up clinically every 3 months until the end of the study at 12 months. Biochemical data will be collected at baseline and every 3 months including hemoglobin, urea, creatinine, albumin, calcium, phosphate, intact parathyroid hormone, brain natriuretic peptide, and high-sensitivity C-reactive protein measured by standard methods. Kt/V values will be also calculated and collected. The inferior vena cava collapsibility index was used to evlaluate volume status[28]. The inferior vena cava was measured by one observer who was blinded to treatment allocation in a supine position during expiration and maximal inspiration, avoiding valsalva-like manoeuvres. The inferior vena cava collapsibility index was calculated using the standard formula: (maximal diameter on expiration - minimal diameter on deep inspiration)/maximal diameter on expiration × 100%. The inferior vena cava collapsibility index, FMD test, and echocardiographic examinations will be performed at baseline, 6 and 12 months.

Participant adherence to the protocol will be monitored by interviews at study check-up visits to promote participant retention and complete follow-up. In order to assess medication adherence, the participants will be asked to take the study medication that is left over for weighing at each clinical visit.

Data collection, management, and monitoring

The data collected at baseline and follow-up visits will fill in the Case Report Forms. Original medical records and informed consents are archived in the participating centers and saved for at least 5 years after the clinical trials finish. All data will be

transferred to the data statistical units for data entry and management with the EpiData3.1 database. The data will be entered independently into the database by two researchers and will be checked separately by different trained researchers. The privacy of the participants is guaranteed. Each participant will receive a participation identity number in this study, with which personal information of participants is labeled in papers. Personal information will be kept in a locked storage unit by one researcher who has will have access to the final trial dataset.

An independent data and safety monitoring board will oversee all aspects of the study. The board will meet periodically during the trial to monitor safety. It will make recommendations on study progress and performance, identify any major adverse outcomes or adverse outcomes due to the therapy, and give advice regarding whether the study should continue or if there should be a protocol change.

Harms

For hemodialysis patients, daily CoQ10 supplementation at doses as high as 1800 mg was safe and well-tolerated [17] [29]. Potential adverse events include gastrointestinal discomfort, loss of appetite, nausea, diarrhea, and rash. In the process of the clinical trial, any severe adverse events must be immediately reported to the ethics committee within 24 h and recorded including the time of occurrence, severity, duration, measures, and outcome.

Statistical methods

The intent-to-treat (ITT) analysis set will be used as the principal analysis for efficacy analyses. All patients who have begun treatment will be included irrespective of their

protocol adherence and continued participation in the study. Patients who complete the study and comply well with the study protocol without major protocol violations will constitute the per-protocol set. The per-protocol analysis will be used as the secondary analysis for efficacy analyses. Missing data will be handled using the last observation carried forward method. Analysis of covariance (ANCOVA) was used to analyze the change from baseline in primary and secondary outcomes adjusted for baseline values and treatment assignment. A 2-tailed $P<0.05$ will be used as the cutoff for statistical significance. All statistical analyses will be performed using Stata software (version 16.0).

Patient and public involvement

The study participants were not involved beyond the standard roles as the subjects of the proposed trial. The public was not involved.

Ethics and dissemination

This study will be performed following the principles of the Declaration of Helsinki. All patients must provide written informed consent (Additional File 2) before undergoing any study-related procedures. The study protocol has been approved by the Medical Ethics Committee for Clinical Trials of Drugs, the 306th Hospital of Chinese PLA. If any significant changes must be made to the protocol, a draft of the new version will be submitted for approval.

We plan to report the trial results for publication in an appropriate journal and to communicate the results at an academic conference. Our final report will follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines [30].

Discussion

CoQ10 is a key component in energy transduction and the antioxidant process [31]. CoQ10 supplementation can be used for the treatment of endothelial dysfunction. For patients with ischemic heart disease, a 1-month treatment of CoQ10 significantly improved FMD from 4.6±0.6 to 7.8± 0.6%, whereas there was no change in the control group [12]. Hamilton et al. found brachial artery FMD was improved by 1% after CoQ10 supplementation for 12 weeks in type 2 diabetic patients [32]. A meta-analysis combined five eligible randomized controlled trials showed that treatment with CoQ10 significantly improved in endothelial function assessed peripherally by flow-mediated dilatation (standard mean difference 1.70, 95% CI: 1.00-2.4, p<0.0001) [11].

The detailed mechanism might involve altering local vascular oxidative stress [12 32]. Tiano's study indicated that improvements of extracellular superoxide dismutase (ecSOD) activity might be related to CoQ10 capability of enhancing endothelial function [12]. In this study, patients with lower levels of ecSOD had greater improvement in endothelial function.

Excessive oxidative stress is highly prevalent and correlated with cardiovascular morbidity and mortality for hemodialysis patients [33]. Excessive oxidative stress might result from due to loss of antioxidants during dialysis and activation of white blood cells triggering the production of reactive oxygen species [34]. Although no study has investigated the effect of CoQ10 supplementation on endothelial function in dialysis patients, increasing evidence has indicated that CoQ10 supplementation can

effectively decrease oxidative stress in this special population [17 35-37].

Regarding cardiac function, for the general population, a meta-analysis showed treatment with CoQ10 had a favorable effect on left ventricular ejection fraction [15]. One randomized controlled trial (Q-SYMBIO trial) [14] and one recent meta-analysis[13] found CoQ10 treatment significantly reduced major adverse cardiovascular events and mortality.

For hemodialysis patients, to our best of knowledge, only two small sample studies focused on the efficacy of supplementation of CoQ10 on cardiac function or biomarker of cardiac function[16 17]. One randomized controlled trial has explored the efficacy of CoQ10 supplementation on cardiac function and found that CoQ10 supplementation decreased left ventricular mass and left ventricular posterior wall as well as interventricular septum thickness, and did not improve diastolic heart function in this special population [16]. However, this study, being a cross-over trial, only had a sample size with a total of 28 participants and short follow-up time with only 8 weeks in each phase and 4-week washout period. Another small sample study [17] indicated that no significant effect of CoQ10 treatment on N-terminal pro-B-type natriuretic peptide (NT-proBNP) was found. However, in the per-protocol analysis, significantly lower levels of NT-proBNP among patients assigned to 1200 mg CoQ10 compared to placebo were found. To date, no study has focused on the effects of CoQ10 treatment on cardiovascular events and mortality.

Based on existing evidence, we hypothesize that the administration of CoQ10 will have a favorable effect on endothelial dysfunction and cardiac function in

hemodialysis patients. Compared with the existing studies, we will first evaluate the effect of CoQ10 treatment on endothelial dysfunction in hemodialysis patients. We will use both echocardiographic parameters and biomarkers of cardiac function to evaluate the cardiac effect of CoQ10. Hard endpoints including cardiovascular events and mortality will be also prespecified outcomes in our study.

However, this is a pilot trial with a small sample size. Hence, this study may not be able to result in significant therapeutic effects. The present trial is a study of great value for it will provide important parameters, which can be used to evaluate the feasibility and safety of the protocol and calculate the sample size for a future trial with a larger sample size to determine whether coenzyme Q10 supplementation confers improved survival and reduced cardiovascular events in hemodialysis patients.

Abbreviations

CoQ10: Coenzyme Q10; A: peak late mitral inflow velocity; ANCOVA: analysis of covariance; E: peak early mitral inflow velocity; e': peak early mitral annulus velocity; E/e': the ratio between peak early mitral inflow velocity (E) and peak early mitral annulus velocity (e'); ecSOD: extracellular superoxide dismutase; FMD: flow-mediated dilation; ITT: Intention-to-treat analysis; LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume; LVM: Left ventricular mass; LVMI: left ventricular mass index; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

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Financial and other competing interests

The authors declare no financial and other competing interests.

Availability of data and materials

The data collected will be made available through requests sent to the lead author.

Contributors

Yongxing Xu and Jianjun Gao conceived the trial idea. Yongxing Xu, Jianjun Gao, Xinlou Li, and Xiaowen Zuo drafted the trial protocol and Enhong Han, Fugui Liang, and Lei Xie contributed to the final version. Yongxing Xu and Jianjun Gao generated the original draft of the manuscript and all authors contributed the final version. Xinlou Li, Xiaowen Zuo, and Huaping Jia provided expert advice in the design of the study. All authors approved the final version for submission.

Figure 1. Study flow chart.

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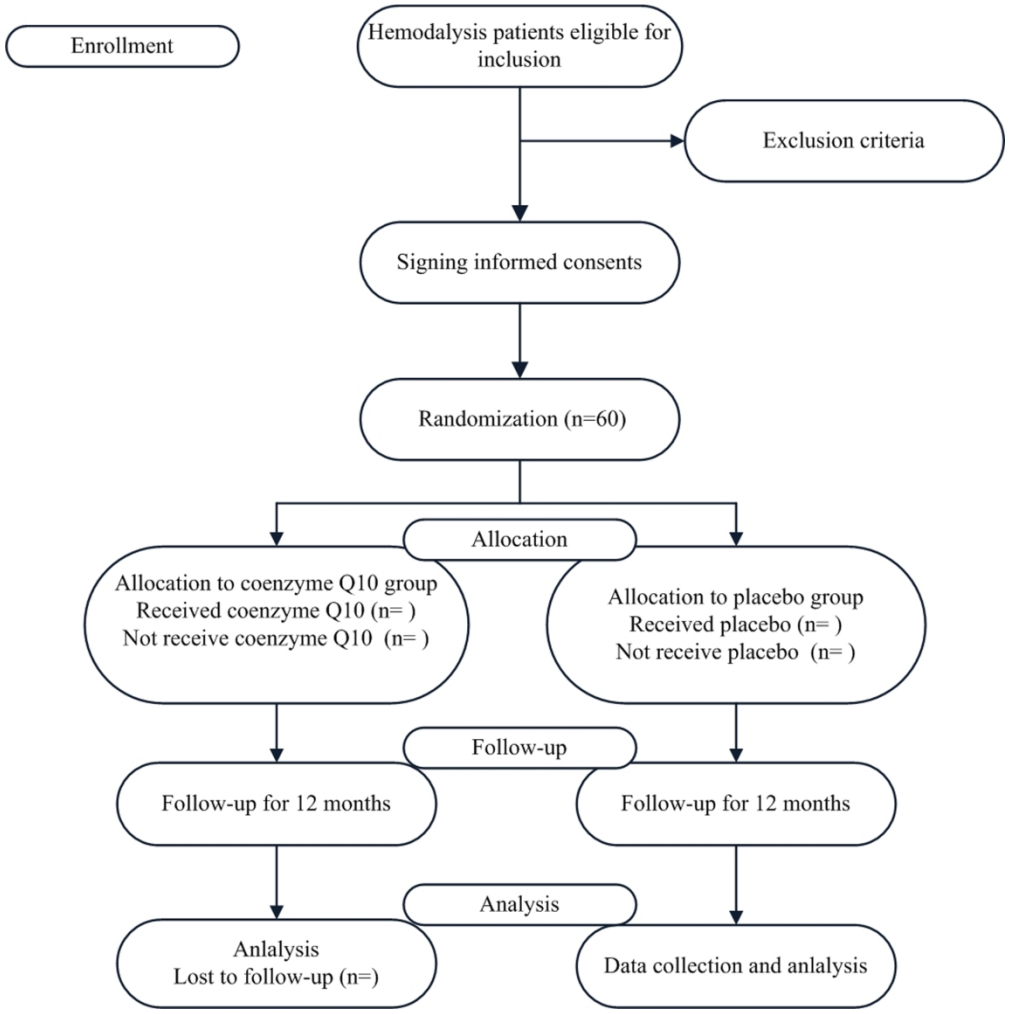

Figure 1. Study flow chart.

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	Page Number on which item is reported
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	Not applicable
Protocol version	3	Date and version identifier	16
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,17
	5b	Name and contact information for the trial sponsor	16
	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	16
	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)	10-11
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

	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	5
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, noninferiority, exploratory)	5,6
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	5
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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
	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)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
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	7-9
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)	5,10

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	6-7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	6
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	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
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	10-11

	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	10-11
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	10-11
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	11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	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)	11-12
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	11
	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	Not applicable
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	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12

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)	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
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	11
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	Not applicable
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	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional File 2
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	Not applicable

*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.

Consent form

（Version 03, November 5, 2018）

Name of subject：_____

Binding address：_____

Telephone number：_____

Dear Mr. / Madam_____:

We sincerely invite you to participate in the clinical trial of "Effects of coenzyme Q10 on endothelial and cardiac function in patients undergoing hemodialysis". This study is conducted in the hemodialysis center of the 306th Hospital of Chinese PLA. Before you agree to participate in this study, please read this consent form carefully. The consent form will provide you with the research background, purpose, method, benefits, the possible risk, and your rights and interests protection during the trial. The information provided in this consent form can help you decide whether to participate in this study or not. If you have any questions, please consult the researchers of the project to ensure that you fully understand the content. If you agree to participate in this study, please sign the consent form and keep a consent form signed by the two sides. This research program has been approved by the ethics committee of the 306th Hospital.

1. Why participate in this study?

Cardiovascular events are highly prevalent. Endothelial and cardiac dysfunction are frequent occurrences in dialysis patients, which are related to higher cardiovascular morbidity and mortality among dialysis patients. Excessive oxidative stress is highly prevalent and also correlated with cardiovascular morbidity and mortality for hemodialysis patients. The excessive oxidative stress might result from due to loss of antioxidants during dialysis and activation of white blood cells triggering the production of reactive oxygen species.

Coenzyme Q10 (CoQ10) is an important in vivo antioxidant. Supplementation with CoQ10 may be of benefit to the general population. Previous studies have demonstrated that oral CoQ10 supplementation improved endothelial dysfunction in the general population. Meanwhile, treatment with CoQ10 can result in improvement in the left ventricular ejection fraction, heart failure, and reducing cardiovascular events and mortality.

Increasing evidence has indicated that CoQ10 supplementation can effectively decrease oxidative stress in dialysis patients. One randomized controlled trial has explored the efficacy of CoQ10 supplementation on cardiac function and found that CoQ10 supplementation decreased left ventricular mass and left ventricular posterior wall as well as interventricular septum thickness, and did not improve diastolic heart function in this special population. Another small sample study indicated that no significant effect of CoQ10 treatment on N-terminal pro-B-type natriuretic peptide (NT-proBNP) was found. However, in the per-protocol analysis, significantly lower levels of NT-proBNP among patients assigned to 1200 mg CoQ10 compared to placebo were found.

Thus, we will conduct this pilot randomized controlled study to evaluate the efficacy and safety of CoQ10 in hemodialysis patients.

This study has been approved by the ethics committee of the 306th Hospital of Chinese PLA. This study complies with the relevant Chinese laws and regulations, the Helsinki declaration and other ethical principles to protect the rights and interests of subjects.

2. Who can take part in the study?

（1）Inclusion criteria

The inclusion criteria are as follows: undergo thrice-weekly hemodialysis for at least 3 months; aged more than 18 and less than 85 years; life expectancy greater than 1 year.

（2）Exclusion criteria

Patients will be excluded if they have any of the following: poor adherence of dialysis or medications; severe systemic or local infection; malignancy; planning to receive kidney transplant within 12 months; hospitalization within 30 days; history of a major atherosclerotic event within 3 months; pregnancy or lactation; current use antioxidant other than vitamin C; use of hemodialysis catheter.

3. How many people participated in the study?

The study is expected to recruit 60 eligible patients. The allocation ratio is 1:1. Participants will be randomized to CoQ10 or placebo group. Both the CoQ10 and placebo will be indistinguishable from each other in shape, size, color and packaging. You may be randomly assigned to the CoQ10 group or placebo group (the probability of being assigned to each group is 50%), but you can only enter only one group for treatment and evaluation. No matter which group you will be assigned, you will be given the appropriate basis and symptomatic treatment for your condition.

4. What is the research procedure?

We will first screen the hemodialysis patients, select the population with inclusion and exclusion criteria, and sign consent form with the patients. After agreeing to be the subject of our study, you will be followed up clinically every 3 months until the end of the study at 12 months. Biochemical data will be collected at baseline and every 3 months including hemoglobin, urea, creatinine, albumin, calcium, phosphate, intact parathyroid hormone, brain natriuretic peptide, and high-sensitivity C-reactive protein measured by standard methods. Kt/V values will be also calculated and collected. FMD test and echocardiographic examinations will be performed at baseline, 6 and 12 months. You will be followed up regularly for 12 months.

5. Possible risks and adverse reactions associated with participation in this study.

(1) The possible side effects and adverse reactions of study drugs

This study will provide you with CoQ10 or placebo. For hemodialysis patients, daily CoQ10 supplementation at doses as high as 1800 mg was safe and well-tolerated according

previous studies. Potential adverse events include gastrointestinal discomfort, loss of appetite, nausea, diarrhea, and rash. If you have any discomfort, new changes in your condition or any unexpected circumstances whether or not related to the drug, you should inform the research doctor in time, and the research doctor will make judgment and medical treatment.

(2) Blood drawing risks: including transient, mild pain, local bruises, mild dizziness in a few people, or extremely rare needle infections.

(3) Other risks: there may also be some other risks, discomfort, drug interactions or adverse reactions that are currently unknown.

6. What are the benefits of participating in this study?

One possible scenario is that you may not directly benefit from this study. Another possible scenario is that your endothelial function or cardiac function will be improved if you are allocated to treatment group if the effects of CoQ10 do exist. But we cannot guarantee this. Although participating in this study may not bring you immediate benefits, your participation may bring benefits to future dialysis patients.

7. If you don't participate in this study, is there any alternative treatment?

If you decide not to participate in this study, you will receive and undergo all usual clinical care activities including thrice-weekly dialysis, regularly monitor cliniacal and laboratory parameters and so on. Your researcher will suggest a treatment plan that suits you. Your researcher will also be happy to explain the possible benefits and risks of other treatments for your disease.

8. The cost of participating in the study

If you participate in this study, you can get free access to CoQ10 or placebo, free measurement for brachial artery flow-mediated dilation, and free echocardiography examination for three times. The above tests and inspection fees shall be borne by this study. However, the medication for the basic treatment and the examination or test items beyond the above instructions shall be at your expense.

9. Management of the occurrence of research-related injuries

If you suffer any adverse events related to this study or cause you any injury during the study period, the researcher will positively treat you and assume relevant responsibilities according to law. The sponsor (investigator) will not be responsible for your medical expenses if you do not comply with the requirements set forth by the investigator under this clinical trial protocol.

10. Voluntary participation and withdrawal from the test

You may choose not to participate in the study, or withdraw consent form from the study after being notified by the researchers at any time without discrimination or retaliation. Your medical treatment and rights will not be affected.

If you cannot make decision immediately, you have enough time to consider, if necessary, you can consult with relatives, friends and other people you trust before making decision.

The investigator may terminate your participation in the study if you require additional diagnosis/treatment, or if you do not follow the study plan, or for any other reasonable reason.

If you decide to withdraw early from a study, it is important that you consult the researchers about what other procedures to follow. During the study period, you may keep in touch with the information related to you in the study.

11. Your personal information will be strictly protected

If you decide to participate in the study, your personal information in and about the study will be kept confidential. Responsible for research physicians and other researchers will use your medical information for research. This information may include your name, address, telephone number, medical history, and information obtained during your study visit. To ensure that the research is conducted in accordance with the regulations, the sponsor, the pharmaceutical administration or the members of the ethics review committee are required to have access to your personal data at the research institute when necessary. Your personal information will not be disclosed when the results of this study are published.

12. Other items

1. For the sake of your health, the researcher may withdraw you from this study without your consent if:

If you continue to participate in this study, the risks may outweigh the benefits;

You do not participate in the study according to the study protocol instructed by the researcher;

The test is terminated prematurely.

2. We still recommend that you take necessary contraceptive measures during the trial if you or your partner during the trial become pregnant, tell the researcher or your physician immediately.

13. Who should you contact if you have any questions or difficulties?

If you have any questions about this study or if you have any discomfort or injury in the course of this study, please contact your research physician: _____。
Telephone:_____。

If you have any questions about your rights and interests during the research, please contact the biomedical ethics committee of our hospital at:_____。

Consent form·Signature page

Subject consent form statement

I have read this consent form and fully understand all the contents.

I have the opportunity to ask questions and all questions have been answered.

I understand that participation in this study is voluntary.

I can choose not to participate in this study, and my medical treatment and benefits will not be affected.

If I need additional treatment, or if I do not follow the study plan, or if there is a research-related injury or for any other reason, the study physician may terminate my continued participation in the study.

I will receive a signed copy of the informed consent.

Name of subject: _____ Signed by legal agent: _____
Signature of the subject: _____ Relationship with subjects: _____
Telephone of subject: _____ Legal representative telephone: _____

Date: Year Month Day Date: Year Month Day
(Note: legal representative's signature is required if subject is incapacitated)

Investigator notification statement

I have informed the subject or his/her legal representative of the purpose, methods, procedures, risks and benefits of the study in detail; Give him/her enough time to read the informed consent form, discuss with others, and answer all questions he/she raises; I have informed the subject of the contact information when encountering problems; I have informed the subject or his/her legal representative that he/she does not need any reason to withdraw from the study at any time during the study.

Name of the investigator: _____

The investigator's signature: _____

Researcher telephone: _____

Date: Year Month Day