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A randomized, open-label, multi-centre trial comparing hemodialysis plus hemoperfusion versus hemodialysis alone in adult patients with end-stage renal disease (HD/HPvsHD): study protocol

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Keywords:	hemodialysis, hemoperfusion, mortality

A randomized, open-label, multi-centre trial comparing hemodialysis plus hemoperfusion versus hemodialysis alone in adult patients with end-stage renal disease (HD/HPvsHD): study protocol

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Wei Lu and Geng-Ru Jiang contributed equally to this paper.

ABSTRACT

Introduction Hemodialysis is the cornerstone treatment for end-stage renal disease (ESRD) patients. However, highly protein-bound or lipophilic toxins such as phenolic and indolic compounds and homocysteine, which are associated with adverse outcomes such as cardiovascular disease, are difficult to remove via hemodialysis. Unlike the diffusive clearance with hemodialysis or convective clearance with hemofiltration, hemoperfusion effectively eliminates highly protein-bound and lipophilic toxins. The HD/HPvsHD (a randomized, open-label, multi-centre trial comparing hemodialysis plus hemoperfusion versus hemodialysis alone in ESRD patients) is the first randomized, open-label, multi-centre therapeutic trial to observe whether hemodialysis plus hemoperfusion is superior to hemodialysis alone in the improvement of patient survival.

Methods and analysis 1364 adult maintenance hemodialysis patients will be enrolled from eleven medical centers in Shanghai and randomized to receive hemodialysis plus hemoperfusion or hemodialysis alone at a 1:1 ratio after 1-month run-in period. In both arms, patients will receive low-flux hemodialysis at a frequency of 2 times a week and hemodiafiltration at a frequency of once a week. In the experiment group, in addition to the treatments in the control arm, hemoperfusion will be conducted once every two weeks using a HA130 resin hemoperfusion apparatus. Follow-up is scheduled at 3, 6, 12, 18, and 24 months after randomization, and will consist: routine physical examinations, standard lab panels (blood routine, liver/kidney functions, coagulation test, etc.), Kt/V, chest X-ray, electrocardiogram, echocardiography, New York Heart Association grading of heart function. The primary outcomes will include 24-month all-cause mortality. Secondary outcomes will include cardiovascular-related mortality, the occurrence of major cardiovascular events and the quality of life assessed by Kidney Disease Quality of Life Short Form.

Ethics and dissemination The protocol has been approved by the Ethical Committees of all eleven participating centres. Study results will be disseminated through peer-reviewed journals and conference presentations.

Trial registration numbers ClinicalTrials.gov Identifier: NCT03227770

Strengths and limitations of this study

- The HD/HPvsHD trial is the first clinical trial that uses mortality as the primary outcome in

ESRD patients receiving hemodialysis plus hemoperfusion versus hemodialysis alone.

- The strength of the proposed study includes relatively long follow-up (24-month), large sample size (n=1,364), and multi centres representing a major Metropolitan area in China.
- A major weakness of the proposed study is the estimation of uremic toxin removal efficiency using only standard tests (e.g., iPTH, hsCRP, β2 macroglobulin and homocysteine). Many relevant protein-bound uremic toxins, including phenolic and indolic compounds, will not be measured (due to practical issues).

Introduction

Despite the advance in blood purification technology and pharmacological treatment, mortality rates of end-stage renal disease (ESRD) patients undergoing renal replacement treatment including hemodialysis and peritoneal dialysis remain high. Cardiovascular disease (CVD) is the leading cause of death in maintenance hemodialysis (MHD) patients.[1] According to the U.S. Renal Data System (USRD), although the mortality rate of MHD patients has dropped by 26% between 1993 and 2012, there was no significant decrease in death caused by CVD.[2] In Japan, the Japanese society for dialysis therapy renal data registry reported that CVD mortality accounts for nearly 40% of all causes of mortality.[3]

There are emerging evidences suggesting that uremic toxins are associated with significant morbidity and mortality in ESRD patients.[4] Based on the physicochemical properties, the European Uremic Toxin Work Group classified uremic toxins into three major categories: 1) small, water-soluble molecules such as urea and creatinine, which can be efficiently removed by hemodialysis; 2) middle molecules larger than 500 Da such as parathyroid hormone (PTH), β2 macroglobulin, and c-reactive protein (CRP), which can be removed by peritoneal dialysis or high-flux hemodialysis or hemofiltration; and 3) protein-bound molecules such as phenolic and indolic compounds, homocysteine (Hcy) that are difficult to remove via hemodialysis or hemofiltration.[5] Recent studies showed that the occurrence of death in ESRD patients is closely associated with the low efficiency of removing middle molecule and protein-bound toxins by using the conventional dialysis therapies.[6-12]

Hemoperfusion allows for the removal of uremic toxins by direct contact with activated charcoal or resin via adsorption and has been one of the preferred methods to enhance poison clearance in intoxication in the clinical practice. Clinical applications of various models of extracorporeal blood purification technologies with the high-to-low clearance rates of large-molecular-weight and protein-bound uremic toxins are in the following order: hemoperfusion > bio-artificial kidney > hemodiafiltration > hemofiltration > hemodialysis.[13, 14] Results from previous small-scaled trials demonstrated that hemoperfusion combined with hemodialysis, conducted by adding a commercially available hemoperfusion apparatus to the dialysis circuit in series with a standard hemodialyzer, is an effective approach to remove not only small water-soluble solutes, but also for middle molecule and protein-bound uremic toxins such as inflammatory cytokines and advanced glycation end products.[13-17] Based on the above results, we hypothesized that

hemodialysis plus hemoperfusion may plausibly improve clinical outcomes of hemodialysis patients, and thus, in this HD/HPvsHD trial, we outlined the first clinical trial to observe whether hemoperfusion combined with hemodialysis is superior to hemodialysis alone in the improvement of survival by significantly reducing the 24-month all-cause and cardiovascular mortality in MHD patients compared with those receiving hemodialysis treatment alone.

Objectives

The primary outcome of the HD/HPvsHD trial is to test if hemodialysis plus hemoperfusion treatment is superior to regular hemodialysis treatment alone in reducing all-cause mortality in MHD patients. We hypothesize that patients receiving hemodialysis plus hemoperfusion treatment have lower rate of all-cause mortality than those receiving hemodialysis alone. The secondary outcome is to test if hemoperfusion combined with hemodialysis treatment is superior to regular hemodialysis treatment in terms of reducing cardiovascular-related mortality and major cardiovascular events (MACEs) as well as improving the quality of life.

Study design

The study will be conducted as a multi-center, open-label, randomized controlled trial. The study will consist of a 1-month run-in period and a 24-month period of intervention and follow-up. The study flow is summarized in Figure 1.

Eligibility criteria

Patients must meet all of the following criteria are eligible:

1） Age at 18-75 years old

2）Regular blood purification treatment at least 3 months before enrolled in this study

3） Standard Kt/V ≥ 1.2

Subjects with one of more of the following conditions will be excluded:

1）Life expectancy <1 year

2）White blood cell count < 4×10^9/L and / or platelet count < 100×10^9/L

3）Cerebral hemorrhage in the past 12 weeks

4）MACEs in the past 8 weeks

5）Severe heart failure (New York Heart Association [NYHA] class III or IV)[18]

6）Active gastrointestinal bleeding, or coagulation dysfunction

7）Malignant tumor

8）Active infection

9）Pregnancy or lactation

10) Participating in clinical trials in the past 3 months

11) Mental disabilities

Sample size calculation

Sample size calculation is based on the following assumptions: 1) α (2-sided) at 0.05; 1) 1-β at 80%; 3) 18% 24-month all-cause mortality in the control arm (subjects receiving hemodialysis and hemodiafiltration only);[1, 2] 4) a reduction of 24-month all-cause mortality by 30% (to 12.6%). Expecting a 15% attrition rate, a total of 682 patients per arm is needed, thus a total of 1364 patients will be needed in this HD/HPvsHD trial.

Randomization and treatment

MHD patients treated in 11 centers across Shanghai, China who met the inclusion criteria will be enrolled into the study. After signing the informed consent, as shown in Table 1., in the 1-month run-in period, the baseline clinical data will be obtained and then patients will be randomized into two treatment arms: experimental group (hemodialysis plus hemoperfusion treatment group) and control group (hemodialysis treatment group). Subjects will be allocated to the 2 arms with a 1:1 ratio using simple randomization. Randomization sequence will be generated by the Clinical Research Unit of Xin Hua Hospital, and distributed to each of the participating centres using opaque envelops for concealment.

Patients randomized to the control group will receive low-flux hemodialysis treatment at a frequency of 2 times a week and online-hemodiafiltration treatment at a frequency of once a week, with each treatment session lasing 4 hours. In the experiment group, in addition to the treatments in the control arm, hemoperfusion will be conducted once every two weeks using a HA130 resin hemoperfusion apparatus containing 130ml resin. During the treatment session,

patients receiving hemodialysis and hemoperfusion for the first 2hrs using a HA130 resin hemoperfusion apparatus containing 130ml resin (Zhuhai Jafron Biomedical Co., Ltd, China) and the blood flow rate maintains between 150-200ml/min. After 2hrs when the hemoperfusion apparatus was depleted, the hemoperfusion cartridge will be removed and the blood went through the low-flux hemodialysis dialyzer alone for the rest 2hrs with the blood flow rate between 200-250ml/min (Figure 2). The dialysate flow rate was 500 ml/min. Heparin, weighing 0.3-0.5mg/kg at the initial 10min and 4-6mg/30min thereafter until the end of treatment session was used. Online-hemodiafiltration will be conducted in the post-dilution mode and the volume of replacement fluid prescription will be 15-20L.

Data collection and management

Clinical data including medical history, physical examination, dialysis regimen, dialysis adequacy determined by standard Kt/V, electrocardiogram (ECG), chest x-ray, echocardiography, heart function rating, the quality of life assessed by the Kidney Disease Quality of Life Short Form (KDQOL-SF),[19] blood routines, liver function, kidney function and other biochemical index of patients will be recorded in an electronic case report file (eCRF) at a secure encrypted database by Viedoc Electronic Data Capture (EDC), which enables an audit trail and is certified by the Good clinical practice (GCP). After verification of the recorded data to source data by one of the executive investigators, recorded data in the eCRF by Viedoc EDC will be exported to an STATA file for further statistical analysis. After the completion of the study, the study database will be locked and data are archived for 10 years in accordance with local policy.

Records will be kept on the occurrence of adverse events, time and cause of patients missing the interview, newly onset or recurrent cardiovascular events and the patients' death. The adverse events are categorized according to the international conference on harmonization guidelines.[20, 21] An outline of the study visits and examinations to be performed is shown in Table 1.

Data analysis

For data analysis, categorical variables will be analyzed using the $\chi 2$ or Fisher's exact test. Continuous variables will be analyzed using Student's *t*-test upon normal distribution, or the Mann-Whitney *U*-test otherwise. Primary outcomes will be analyzed using the Kaplan-Meier method followed by the log-rank test. Multivariate Cox regression will be used to analyzed

factors that could influence all-cause mortality and CVD mortality after the adjustment for multiple relevant traditional and uremia-related risk factors for mortality. Data were analyzed on an intention-to-treat basis. All statistical analyses will be conducted using STATA (Version 14.0; Stata Corporation, College Station, Texas, US).

Ethics and dissemination

The protocol has been approved by Ethical Committee of eleven participating centres and has been assigned the following protocol ID: NCT03227770. Substantive protocol amendments will be reported, reviewed and approved by the local medical ethical committee before application. Results of this study will be presented at national and international scientific meetings, and publications will be submitted to peer-reviewed journals.

Discussion

There are several unique features of the HD/HPvsHD trial compared with prior trials of combined hemoperfusion and hemodialysis. The HD/HPvsHD trial is the first clinical trial that uses mortality as the primary outcome in ESRD patients receiving hemodialysis plus hemoperfusion versus hemodialysis alone. As hemoperfusion combined with conventional hemodialysis is an effective approach to remove not only small water-soluble solutes, but also for middle molecule and protein-bound uremic toxins, it may plausibly improve patient prognosis, thus we hypothesized that the combination treatment of hemodialysis plus hemoperfusion might reduce all-cause mortality of MHD patients. Secondly, the strength of the proposed study includes relatively long follow-up (24-month), large sample size (n=1,364), and multi centres representing a major Metropolitan area in China.

The design of this study has the limitation that we only select several representative middle molecules and protein-bound uremic toxins such as iPTH, hsCRP, β2-macroglobulin and homocysteine and tested the removal efficiency of these uremic toxins by both treatment arms. Testing for protein-bound uremic toxins such as phenolic and indolic compounds are unavailable in the clinical setting, which is one main limitation of this study. Additionally, hemoperfusion treatment will have potential complications and side effects such as transient leukopenia, minor reduction in fibrinogen and fibronectin and blood reactions to biocompatible materials.

In summary, the HD/HPvsHD trial will present an opportunity to assessing the efficacy of hemodialysis plus hemoperfusion in improving the survival of MHD patients. Results from this trial may provide guidance to the optimization of blood purification therapy in MHD patients.

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Authors' contributions

Wei Lu and Geng-Ru Jiang were involved in conception and trial design and in drafting of the article.

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Competing interests statement

None.

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Tables

Table 1. Study visits of the HD/HPvsHD trial

	V1	V2	V3	V4	V5	V6	V7
	-1M	0M	3M±14d	6M±14d	12M±14d	18M±14d	24M±14d
Consent form	×						
Medical hisotory	×	×	×	×	×	×	×
Physical examination	×	×	×	×	×	×	×
Eligibility		×					
Dialysis regimen	×	×	×	×	×	×	×
Blood routine and coagulation test	×	×	×	×	×	×	×
*Blood chemistry	×			×	×	×	×
Serum iPTH	×			×	×	×	×
Serum hsCRP	×			×	×	×	×
Serum β_2-MG	×			×	×	×	×
Serum Hcy	×			×	×	×	×
Standard Kt/V	×			×	×	×	×
electrocardiogram	×				×		×
Chest X-ray	×				×		×
Echocardiography	×				×		×
Heart function rating	×	×	×	×	×	×	×
KDQOL-SF		×			×		×
Adjustment of dialysis regimen		×	×	×	×	×	×
Comorbidity	×	×	×	×	×	×	×
Medications	×	×	×	×	×	×	×
Adverse events		×	×	×	×	×	×

*: Blood chemistry include liver function series (total bilirubin, direct bilirubin, alanine aminotransferase, aspartate aminotransferase, serum total protein, albumin, γ-glutamyltransferase and alkaline phosphatase), renal functions (blood urea nitrogen and creatinine), electrolytes (natrium, potassium, calcium and phosphate), glucose, creatinine kinase and its isoform CK-MB, troponin I, myohemoglobin, lactate dehydrogenase, α–hydroxybutyrate dehydrogenase, pro b-type natriuretic peptide.

Figure legends

Figure 1. Study flow diagram of the HD/HPvsHD trial.

Figure 2. The schematic diagram of hemodialysis plus hemoperfusion treatment.

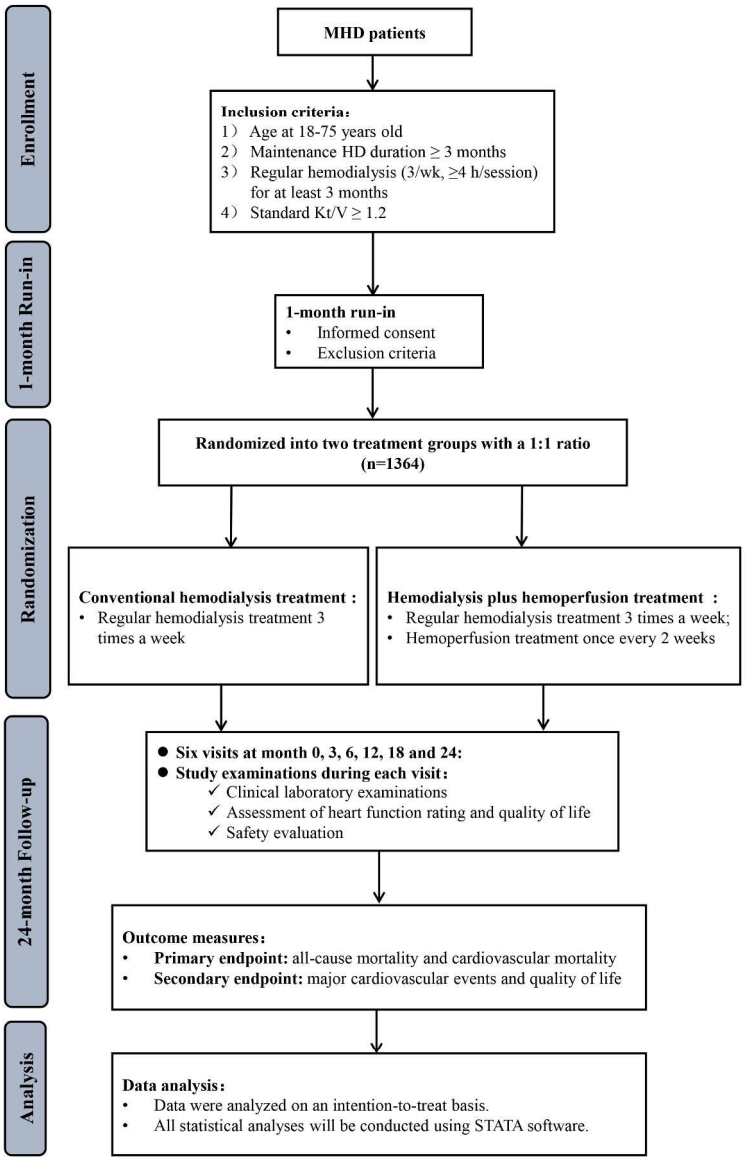

Figure 1. Study flow diagram of the HD/HPvsHD trial.

190x302mm (300 x 300 DPI)

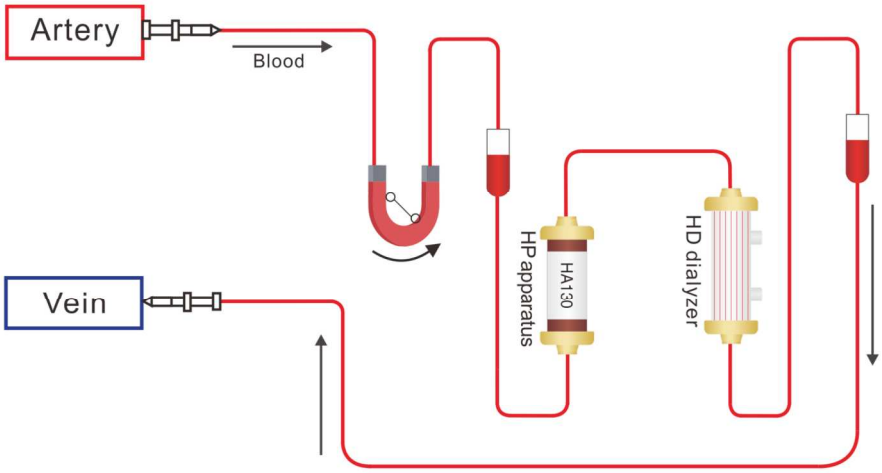

Figure 2. The schematic diagram of hemodialysis plus hemoperfusion treatment.

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Renal medicine
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Wei Lu and Geng-Ru Jiang contributed equally to this paper.

ABSTRACT

Introduction Hemodialysis (HD) is the cornerstone treatment for end-stage renal disease (ESRD) patients. However, highly protein-bound or large molecular weight uremic toxins such as phenolic and indolic compounds and homocysteine, which are associated with adverse outcomes such as cardiovascular disease of ESRD patients, are difficult to remove via HD but can be effectively eliminates by hemoperfusion (HP). The proposed trial (referred to as HD/HPvsHD below) is a randomized, open-label, multi-centre trial comparing HD plus HP versus HD alone in adult patients with ESRD. The primary endpoint measure is all-cause mortality.

Methods and analysis We plan to enroll 1364 maintenance hemodialysis patients from eleven medical centers in Shanghai. Participants will be randomized to receive HD plus HP or HD alone at a 1:1 ratio after 1-month run-in period. In both arms, patients will receive low-flux HD at a frequency of 2 times a week and hemodiafiltration (HDF) at a frequency of once a week. In the intervention group, subjects also received HP once every two weeks. Follow-up is scheduled at 3, 6, 12, 18, and 24 months after randomization, and will consist the following: routine physical examinations, standard lab panels (blood routine, liver / residual kidney functions, tests of the coagulation system, etc.), dialysis adequacy (standard Kt/V), chest X-ray, electrocardiogram, echocardiography, heart function rating. Adverse events will be assessed according to the international conference on harmonization guidelines. The primary outcome is 24-month all-cause mortality. Secondary outcomes will include cardiovascular-related mortality, the occurrence of major cardiovascular events and the quality of life.

Ethics and dissemination The study protocol has been approved by the Ethical Committees of all eleven participating centres. Clinical Research Unit of Xin Hua Hospital will oversee the study. The results will be presented at national and international academic meetings, and submitted to peer-reviewed journals for publications.

Trial registration numbers ClinicalTrials.gov Identifier: NCT03227770

Protocol version identifier 2.0

Strengths and limitations of this study

- The HD/HPvsHD trial is the first clinical trial that uses mortality as the primary outcome in ESRD patients receiving HD plus HP versus HD alone.
- The strength of the proposed study includes relatively long follow-up (24-month), large sample size (n=1,364) and multiple centres representing a major Metropolitan area in China.

- A major weakness of the proposed study is the estimation of uremic toxin removal efficiency using only standard tests (e.g., iPTH, hsCRP, □□-macroglobulin and homocysteine). Many relevant protein-bound uremic toxins, including phenolic and indolic compounds, will not be measured (due to practical issues).

Introduction

Despite the advance in blood purification technology and pharmacological treatment, mortality rates of end-stage renal disease (ESRD) patients undergoing renal replacement treatment including hemodialysis (HD) and peritoneal dialysis remain high. Cardiovascular disease (CVD) is the leading cause of death in maintenance hemodialysis (MHD) patients.[1] According to the U.S. Renal Data System (USRD), although the mortality rate of MHD patients has dropped by 26% between 1993 and 2012, there was no significant decrease in death caused by CVD.[2] In Japan, the Japanese society for dialysis therapy renal data registry reported that CVD mortality accounts for nearly 40% of all causes of mortality.[3]

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Hemoperfusion (HP) allows for the removal of uremic toxins by direct contact with activated charcoal or resin via adsorption and has been one of the preferred methods to enhance poison clearance in intoxication in the clinical practice. Clinical applications of various models of extracorporeal blood purification technologies with the high-to-low clearance rates of large-molecular-weight and protein-bound uremic toxins are in the following order: hemoperfusion > bio-artificial kidney > hemodiafiltration > hemofiltration > hemodialysis.[13, 14] Results from previous small-scaled trials demonstrated that HP combined with HD, conducted by adding a commercially available hemoperfusion apparatus to the dialysis circuit in series with a standard hemodialyzer, is an effective approach to remove not only small water-soluble solutes, but also for middle molecule and protein-bound uremic toxins such as inflammatory cytokines and advanced glycation end products.[13-17] Based on the above results, we hypothesized that HD plus

HP may plausibly improve clinical outcomes of hemodialysis patients, and thus, in this HD/HPvsHD trial, we outlined the first clinical trial to observe whether HP combined with HD is superior to HD alone in the improvement of survival by significantly reducing the 24-month all-cause and cardiovascular mortality in MHD patients compared with those receiving HD alone.

Objectives

The primary outcome of the HD/HPvsHD trial is to test if HD plus HP treatment is superior to regular HD alone in reducing all-cause mortality in MHD patients. We hypothesize that patients receiving HD plus HP treatment have lower rate of all-cause mortality than those receiving HD alone. The secondary outcome is to test if HD plus HP treatment is superior to regular HD treatment in terms of reducing cardiovascular-related mortality and major cardiovascular events (MACEs) as well as improving the quality of life.

Study design

The study will be conducted as a multi-center, open-label, randomized controlled trial. The study will consist of a 1-month run-in period and a 24-month period of intervention and follow-up. The study flow is summarized in Figure 1.

Eligibility criteria

Patients must meet all of the following criteria are eligible:

1) Age at 18-75 years old;
2) Regular blood purification treatment at least 3 months before enrolled in this study;
3) Standard Kt/V $\geq$ 1.2.

Subjects with one of more of the following conditions will be excluded:

1) White blood cell count < 4×10^9/L and / or platelet count < 100×10^9/L;
2) Cerebral hemorrhage in the past 12 weeks;
3) MACEs in the past 8 weeks;
4) Severe heart failure (New York Heart Association [NYHA] class III or IV);[18]
5) Active gastrointestinal bleeding, or coagulation dysfunction;
6) Malignant tumor;

7) Active infection;
8) Pregnancy or lactation;
9) Participating in clinical trials in the past 3 months;
10) Mental disabilities.

Sample size calculation

Sample size calculation is based on the following assumptions: 1) □ (2-sided) at 0.05; 1) 1-□ at 80%; 3) 18% 24-month all-cause mortality in the control arm;[1, 2] 4) a reduction of 24-month all-cause mortality by 30% (to 12.6%). Expecting a 15% attrition rate, a total of 682 patients per arm is needed, thus a total of 1364 patients will be needed in this HD/HPvsHD trial. There are currently around 2000 MHD patients meeting eligibility criteria in total in the eleven participating centers of this HD/HPvsHD trial, which demonstrated that we are capable to achieving adequate participant enrolment to reach target sample size.

Randomization and treatment

MHD patients treated in 11 centers across Shanghai, China who met the inclusion criteria will be enrolled into the study. After signing the informed consent, as shown in Table 1., in the 1-month run-in period, the baseline clinical data will be obtained and then patients will be randomized into two treatment arms: experimental group (HD plus HP treatment group) and control group (HD treatment group). Subjects will be allocated to the 2 arms with a 1:1 ratio using simple randomization. Randomization sequence will be generated by the statistics research section (SRS) of the Clinical Research Unit of Xin Hua Hospital, and distributed to each of the participating centres using opaque envelops for concealment.

Patients randomized to the control group will receive low-flux HD treatment at a frequency of 2 times a week and online-hemodiafiltration (HDF) treatment at a frequency of once a week, with each treatment session lasing 4 hours. In the experiment group, in addition to the treatments in the control arm, HP will be conducted once every two weeks using a HA130 resin hemoperfusion apparatus containing 130ml resin. During the treatment session, patients receiving HD and HP for the first 2hrs using a HA130 resin hemoperfusion apparatus containing 130ml resin (Zhuhai Jafron Biomedical Co., Ltd, China) and the blood flow rate maintains between 150-200ml/min. After 2 hours when the HP apparatus was depleted, the HP cartridge

will be removed and the blood went through the low-flux HD dialyzer alone for the rest 2 hours with the blood flow rate between 200-250ml/min (Figure 2). The dialysate flow rate was 500 ml/min. Heparin, weighing 0.3-0.5mg/kg at the initial 10min and 4-6mg/30min thereafter until the end of treatment session was used. Online-HDF will be conducted in the post-dilution mode and the volume of replacement fluid prescription will be 15-20L.

Data collection and management

Clinical data including medical history, physical examination, dialysis regimen, dialysis adequacy determined by standard Kt/V, electrocardiogram (ECG), chest x-ray, echocardiography, heart function rating, the quality of life assessed by the Kidney Disease Quality of Life Short Form (KDQOL-SF),[19] blood routines, liver function, residual kidney function [20] and other biochemical index of patients will be recorded in an electronic case report file (eCRF) at a secure encrypted database by Viedoc Electronic Data Capture (EDC), which enables an audit trail and is certified by the Good Clinical Practice (GCP). An outline of the study visits and examinations to be performed is shown in Table 1.

After verification of the recorded data to source data by the data monitoring committee (DMC), recorded data in the eCRF by Viedoc EDC will be exported to an STATA file for further statistical analysis. Any missing data, data out of pre-limits and possible duplication for each end point will be queried and internally validated by the DMC before locking the database. The DMC is independent of the study investigators and has no competing interests. After the completion of the study, the study database will be locked and data are archived for 10 years in accordance with local policy. Records will be kept on the occurrence of adverse events, time and cause of patients missing the interview, newly onset or recurrent cardiovascular events and the patients' death. An interim analysis will be performed on primary end-point when half of the patients have been randomized and have completed the 24-month follow-up. The interim analysis will be performed by the Clinical Research Unit of Xin Hua Hospital, who has no competing interests, and will decide whether to continue with the trial.

Adverse events monitoring

The adverse events (AE) are categorized according to the international conference on harmonization guidelines.[21, 22] The causal relationship to study drug is determined by the

physician. All AEs will be collected and recorded after the patient has provided consent and is included in the trial. An AE which meets the criteria for a serious adverse event (SAE) between study enrolment and hospital discharge will be reported to the DMC and trial management committee within 24 hours. An SAE report should be completed for any event where doubt exists regarding its status of seriousness. All SAEs should be followed to resolution or stabilization. Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug. If patients suffer harm as a result of their participation in the trial, there will receive free treatment and sufficient compensation.

Data analysis

For data analysis, categorical variables will be analyzed using the □2 or Fisher's exact test. Continuous variables will be analyzed using Student's *t*-test upon normal distribution, or the Mann-Whitney *U*-test otherwise. Primary outcomes will be analyzed using the Kaplan-Meier method followed by the log-rank test. Multivariate Cox regression will be used to analyzed factors that could influence all-cause mortality and CVD mortality after the adjustment for multiple relevant traditional and uremia-related risk factors for mortality. Data were analyzed on an intention-to-treat basis. All statistical analyses will be conducted using STATA (Version 14.0; Stata Corporation, College Station, Texas, US).

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

The protocol has been approved by Ethical Committee of eleven participating centres and has been assigned the following protocol ID: NCT03227770. Substantive protocol amendments will be reported, reviewed and approved by the local medical ethical committee before application. The study may be subject to inspection and audit by Clinical Research Unit of Xin Hua Hospital to ensure adherence to the guidelines of GCP. The frequency of monitoring visits will be

determined by the site enrolment rate. On study completion, the study monitor will conduct a study termination visit.

Results of this study will be presented at national and international scientific meetings, and publications will be submitted to peer-reviewed journals.

Consent and confidentiality

This trial will be performed in accordance with the Declaration of Helsinki. Each patient will need to agree with a fully informed consent form and sign it. All laboratory specimens, data collection, reports, administrative forms and the process itself will be identified by a coded ID to maintain patient confidentiality. All records that include the name or personal identifier will be stored separately from records identified by ID. Datasets on the website will be protected by password. The principal investigators will need to sign agreements to maintain the confidentiality of all patients. Study information relating to any patient will not be released externally without the written permission of the patient.

Discussion

There are several unique features of the HD/HPvsHD trial compared with prior trials of combined hemoperfusion and hemodialysis. The HD/HPvsHD trial is the first clinical trial that uses mortality as the primary outcome in ESRD patients receiving HD plus HP treatment versus receiving HD treatment alone. As HD combined with HP is an effective approach to remove not only small water-soluble solutes, but also for middle molecule and protein-bound uremic toxins, it may plausibly improve patient prognosis, thus we hypothesized that the combination treatment of HD plus HP might reduce all-cause mortality of MHD patients. Secondly, the strengths of the proposed study include relatively long follow-up (24-month), large sample size (n=1,364) and multiple centres representing a major Metropolitan area in China.

The design of this study has the limitation that we only select several representative middle molecules and protein-bound uremic toxins such as iPTH, hsCRP, □2-macroglobulin and homocysteine and tested the removal efficiency of these uremic toxins by both treatment arms. Testing for protein-bound uremic toxins such as phenolic and indolic compounds are unavailable in the clinical setting, which is one main limitation of this study. Additionally, HP treatment will have potential complications and side effects such as transient leukocytopenia and

thrombocytopenia, minor reduction in fibrinogen and fibronectin and blood reactions to biocompatible materials.[23] Thus, in the experiment group, in addition to the HD treatments in the control arm, HP will be added once every two weeks. Third, due to the higher cost (both HD-related equipment and accessories) associated with high-flux HD, low-flux HD twice weekly plus HDF once weekly is a widely-used protocol across China. Thus, the results of the proposed trial must be interpreted with caution when extrapolating to US and Europe, where the conventional protocol is high-flux HD 3 times per week. Regardless, the results of the proposed trial will provide useful information that could potentially change the medical practice in the US and Europe if additional trials are conducted in subjects receiving high-flux HD 3 times per week. In summary, the HD/HPvsHD trial will present an opportunity to assessing the efficacy of hemodialysis plus hemoperfusion in improving the survival of MHD patients. Results from this trial may provide guidance to the optimization of blood purification therapy in MHD patients.

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Authors' contributions

Wei Lu and Geng-Ru Jiang were involved in conception and trial design and in drafting of the article. The HD/HPvsHD trial Group were participating in the trial.

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Competing interests statement

There are no competing interests for any author.

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Tables

Table 1. Study visits of the HD/HPvsHD trial

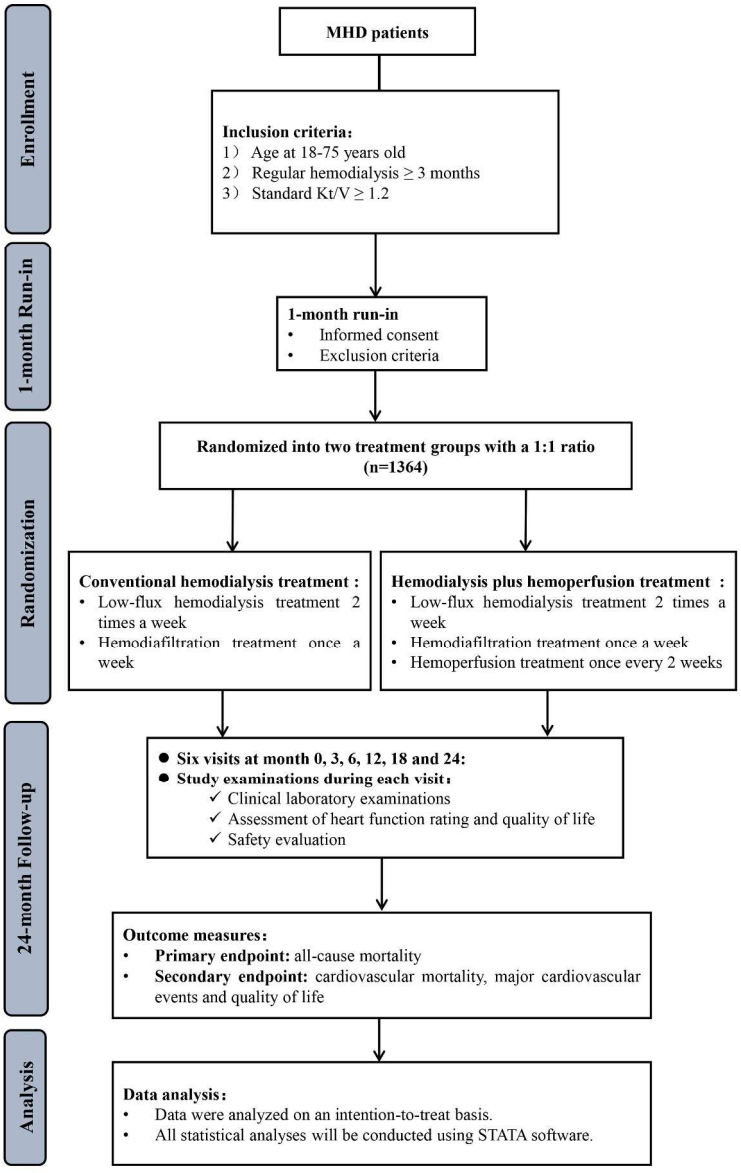
	V1	V2	V3	V4	V5	V6	V7
	-1M	0M	3M±14d	6M±14d	12M±14d	18M±14d	24M±14d
Consent form	×						
Medical history	×	×	×	×	×	×	×
Physical examination	×	×	×	×	×	×	×
Eligibility		×					
Dialysis regimen	×	×	×	×	×	×	×
Blood routine and coagulation test	×	×	×	×	×	×	×
*Blood chemistry	×			×	×	×	×
Residual kidney function	×						
Serum iPTH	×			×	×	×	×
Serum hsCRP	×			×	×	×	×
Serum β_2-microglobulin	×			×	×	×	×
Serum Hcysteine	×			×	×	×	×
Standard Kt/V	×			×	×	×	×
electrocardiogram	×				×		×
Chest X-ray	×				×		×
Echocardiography	×				×		×
Heart function rating	×	×	×	×	×	×	×
KDQOL-SF		×			×		×
Adjustment of dialysis regimen		×	×	×	×	×	×
Comorbidity	×	×	×	×	×	×	×
Medications	×	×	×	×	×	×	×
Adverse events		×	×	×	×	×	×

*: Blood chemistry include liver function series (total bilirubin, direct bilirubin, alanine aminotransferase, aspartate aminotransferase, serum total protein, albumin, γ-glutamyltransferase and alkaline phosphatase), serum blood urea nitrogen and creatinine, electrolytes (natrium, potassium, calcium and phosphate), glucose, creatinine kinase and its isoform CK-MB, troponin I, myohemoglobin, lactate dehydrogenase, □□hydroxybutyrate dehydrogenase, pro b-type natriuretic peptide.

Figure legends

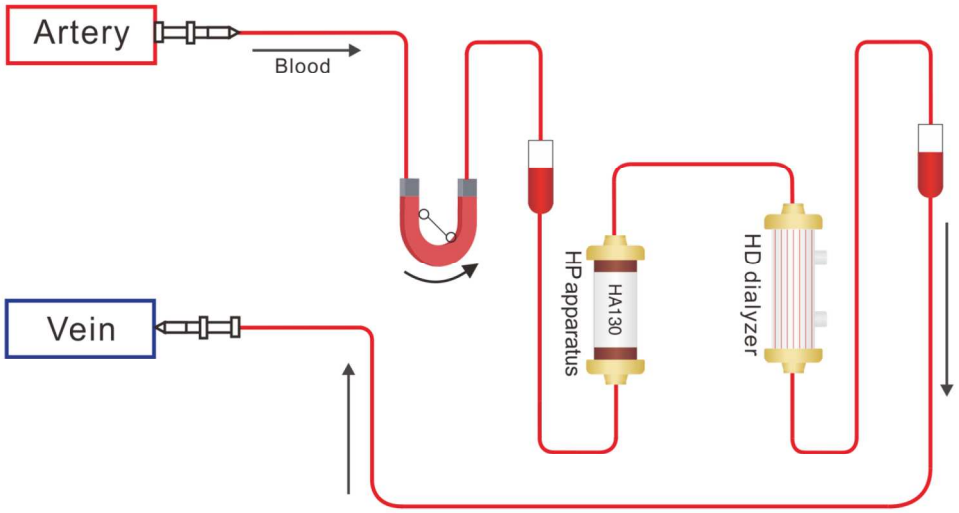
Figure 1. Study flow diagram of the HD/HPvsHD trial.

Figure 2. A schematic diagram of hemodialysis plus hemoperfusion treatment.



Study flow diagram of the HD/HPvsHD trial.

190x299mm (300 x 300 DPI)



A schematic diagram of hemodialysis plus hemoperfusion treatment.

175x100mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Line 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Line 122
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Line 123
Funding	4	Sources and types of financial, material, and other support	Line 471-473
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Line 4, Line 15
	5b	Name and contact information for the trial sponsor	Line 471-473
	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	Line 467-469
	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)	Line 58-61

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	line 179 - 188
	6b	Explanation for choice of comparators	line 180 - 188
Objectives	7	Specific objectives or hypotheses	line 188 - 197
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)	line 199 - 205
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	line 280 - 283
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)	line 212 - 274
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	line 285 - 323
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	line 282 - 283
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	line 199 - 205
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)	line 285 - 323, Figure 2

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	line 276-283
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	line 282-283
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	line 290-293
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	line 290-293
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	line 290-298
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A (open-label)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A (open-label)
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	line 326-333
	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	N/A

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	line 334 – 338
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	line 376-383
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	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)	line 376-383
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	line 334 ~~334~~ – 338
	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	line 342-345
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	line 348 – 373
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	line 389 - 392
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	line 388 - 387.
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)	line 387~388

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	line 397
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	line 398-400
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	line 477-478.
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	line 334-338
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	line 372-373
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	line 393-394
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	line 404-406
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
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	N/A

*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.