A Postbiotics Improves the Quality of Life and Nutritional Status in Elderly Hemodialysis Patients （Bio-EHD）

Research Program

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Sponsor: The Department of Nephrology, Peking University First Hospital
Project Leader: Chen, Yuqing
Department: The Department of Nephrology, Peking University First Hospital
Telephone: 010-82572254
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Content

1. Background

High prevalence of chronic kidney disease (CKD) is a threat to human health, and maintenance hemodialysis is an important treatment for ESRD patients. However, even after hemodialysis and conventional treatment, CKD patients still often suffer from the complications like malnutrition, anemia, calcium and phosphorus metabolism disorders, which increase morbidity and mortality rate[1-2]. How to reduce complications and improve prognosis of CKD patients is now a significant issue in the prevention and treatment of CKD worldwide. Intestinal flora imbalance has a high prevalence in CKD patients and is closely associated with malnutrition, anemia, calcium and phosphorus metabolism disorders and other complications, affecting patients' quality of life and long-term prognosis[3].

Due to increasing level of uremic toxins, perennial diet control, antibiotic application and oral iron, the symbiotic relationship between host and microorganism of the CKD patients is broken, which leads to intestinal flora imbalance and manifests as a significant decrease in anaerobic probiotics and a significant increase in aerobic harmful flora[4]. Intestinal flora could produce an excess of uremic toxins such as indoxyl sulfate (IS), p-cresyl sulfate(PCS), trimethylamine N-oxide(TMAO), acetanilide and lipopolysaccharide during the fermentation of proteins and amino acids. Physiologically, these enterogenous urotoxins are secreted through renal tubules and cleared by the kidneys, however, CKD patients have renal insufficiency, and these medium molecular toxins are difficult to be cleared by dialysis, resulting in the accumulation of toxins in the blood. A lot of clinical studies show that urotoxins not only promote the occurrence and development of CKD but also produce effect on anemia, malnutrition, bone-metabolism disorder, cardio- and cerebrovascular

complications. Because of increasing intestinal urotoxin, damage of intestinal mucosal epithelial barrier, delayed intestinal drainage, intestinal mucosa edema and ischemia, and the nutrient absorption of CKD patients are reduced. In addition, with a low protein diet, CKD patients are prone to malnutrition. Intestinal flora imbalance, translocation and accumulation of enterogenous urotoxin and malnutrition can form a vicious cycle, leading to poor prognosis of patients[6]. Current studies show that polyamine toxoid can inhibit EPO activity and affect erythropoiesis[7]. IS and PCS can induce osteodystrophy and disordered bone metabolism by several mechanism[8], like promoting parathyroid hormone resistance, inhibiting osteoclast function and osteoblast differentiation. Besides, IS and PCS can accelerate renal fibrosis by inhibiting the expression of kidney protective genes (such as Klotho), activating renin-angiotensin-aldosterone system, mediating oxidative stress and activating inflammatory response[9]. The increasing plasma TMAO levels can lead to renal atherosclerosis[10]. IS, PCS and TMAO can damage vascular endothelial cells through various pathways, promote vascular calcification and atherosclerosis, and lead to cardio- and cerebrovascular events[11-12]. Therefore, preventing intestinal flora imbalance and reducing the production and accumulation of urotoxin can provide new therapeutic approaches for anemia, malnutrition, disordered bone metabolism and other complications in CKD and MHD patients.

Currently, there are several therapeutic approaches for intestinal flora imbalance, including ①High dietary fiber, low animal protein and low fat diet; ② Toxin adsorbents like activate carbon; ③Fecal transplantation; ④Micro ecological preparations like probiotics, prebiotics, synbiotics and postbiotics. CKD and MHD patients need to restrict potassium intake, which will inevitably lead to the reduction of fructose and cellulose, so that simply diet adjustment has little effect on the improvement of CKD. Activate carbon is considered to be an intestinal purifier which

can effectively reduce the absorption of gastrointestinal toxins in acute poisoning, but long-term application can cause gastrointestinal adverse reactions such as nausea and vomiting, which causes intestinal peristalsis dysfunction and even intestinal obstruction. Fecal transplantation is used to transfer of microbial flora extracted from the stool of healthy people into the intestines of patients, but it's still difficult to ensure the long-term stability and safety of the donor stool and find a suitable donor. According to current studies, using micro ecological preparations to increase probiotics can play a role in prevention and treatment of CKD.

Probiotics are a kind of active microorganisms which are beneficial to human body and can improve the structure of intestinal flora. Studies have confirmed that bifidobacterium preparations can effectively bind to human intestinal mucosal epithelial cells to build biological barriers after oral entry into the intestine. Bifidobacterium can produce extracellular glycosidase to degrade complex polysaccharides so that it inhibits pathogenic bacteria significantly[13]. Lactobacillus and bifidobacterium formulations have been shown to reduce PCS and IS levels in patients and reduce systemic inflammatory responses[14]. Moreover, bifidobacterium can promote intestinal peristalsis and drainage of intestinal toxic metabolite. Prebiotics are organic substances that are not digested and absorbed by the host but can promote the proliferation and metabolism of beneficial bacteria in the body. The intestinal bifidobacterium can increase significantly when dietary adjustment is combined with the intake of prebiotics (such as inulin) [15]. Symbiotic is a combination of probiotics and prebiotics and it can both supplement probiotics and promote the growth and survival of probiotics[16]. Therefore, the application of micro ecological preparation, supplementing probiotics, prebiotics and symbiotics can stimulate and regulate intestinal flora, alleviate CKD disease to a certain extent, and improve the quality of life of patients, which is of great significance. There are several

disadvantages of living bacteria components such as high requirement for preservation and transportation, potential safety risks, intolerance to stomach acid, and non-use with antibiotics. In contrast, inactivated probiotics have their unique advantages. Recently, inactivated probiotics preparations attract more and more attention and are known as probiotics. Probiotics are those bioactive compounds produced by probiotics when they consume prebiotics, including short-chain fatty acid, lipolyaccharide, enzyme, exopolysaccharide, cell-free supernatant, inactivated probiotic bacteria or cell fragments (e.g. cell wall fragments, bacterial lysates, etc.) and other metabolites (e.g. vitamins, amino acids, etc.). Short-chain fatty acids in postbiotic are an important energy source for cells and play a vital role in the renewal of cells in the intestinal wall. They help to maintain the integrity of the intestinal barrier and can be used as an adjunct therapy of inflammatory bowel disease and other digestive disorders. Postbiotics can regulate microbiota directly and indirectly and inhibit growth of pathogenic microorganism, for example, bacteriocin secreted by lactic acid bacteria can inhibit the growth of intestinal pathogens.

MHD patients have a high incidence of intestinal flora disorders. Malnutrition and other complication progression causing by intestinal flora disorders in MHD patients currently lacks perfect treatment options, affecting their life quality and prognosis, increasing the hospitalization rate and medical costs. In elderly patients, malnutrition, intestinal dysfunction, and reduced quality of life are more prominent. The purpose of the study is to investigate the effects of exogenous supplementation of intestinal probiotic metabolites on life quality, nutrition, intestinal flora and urotoxins in elderly MHD patients, and to explore the feasibility and effect of new treatment methods.

2. Purpose

The purpose of the trial is to explore the effect of exogenous postbiotics supply on the quality of life and nutritional status of elderly hemodialysis patients.

3. Method

3.1 Design

The study is a multicenter prospective open labeled randomized controlled trial. Elderly patients with age ⩾65 years and received hemodialysis treatment for more than 6 months will be included in the study. After enrollment, the patients will be educated about how to keep diet records and then they will be randomly divided into group A and group B in a 1:1 ratio. The patients in group A will receive oral postbiotics and group B receive placebo. The treatment period is 12 weeks. Subsequently, group A ends the trial, and group B enters the remedial treatment period, receiving oral postbiotics, and the treatment cycle is 12 weeks. At 0, 12^{th} and 24^{th} week, quality of life score, nutrition evaluation, diet status and gastrointestinal symptom score of patients will be evaluated. The stool and serum of patients will be collected for intestinal flora and urotoxins IS, PCS and TMAO measurements. The improvement of biochemical indicators will be evaluated. The biochemical indicators include blood creatinine, urea nitrogen, albumin, prealbumin, hemoglobin, blood calcium, blood phosphorus, whole parathyroid hormone, blood potassium, C-reactive protein, ferritin, transferrin saturation, et al. During the whole intervention process, patients will be encouraged to maintain intake of protein and fiber and keep a diet log.

During the intervention process, the process of hemodialysis and other diagnosis and treatment behaviors will be performed according to clinical routine.

3.2 Site for Data Collection

The medical history collection, questionnaire, and anthropometric measurements of the enrolled patients will be conducted within the first week of the study, and then once each week at the 12th and 24th weeks. The questionnaire will be completed by specially trained staff. Stool and serum samples will be collected at weeks 0, 12^{th} and 24^{th} for intestinal flora and urotoxin determination. The routine biochemical indicators of the patients will be collected, including serum creatinine, urea nitrogen, albumin, prealbumin, hemoglobin, serum calcium, blood phosphorus, total parathyroid hormone, serum potassium, carbon dioxide, C-reactive protein, ferritin, transferrin saturation, et al.

3.3 Population

3.3.1 Participation Criteria

Inclusion criteria:

Investigators must maintain a screening log of all potential study candidates that includes limited information about the potential candidate, date, and outcome of the screening process (e.g. enrolled into study, reason for ineligibility, or refused to participate). After signing an informed consent form, potential patients must meet all of the criteria:

1. Age ⩾65 years.
2. Receiving maintenance hemodialysis therapy for at least 6 months.

Exclusion criteria:

The following exclusion criteria must NOT be present for each subject:

1. Subjects with medical history that might affect oral feeding (e.g. intestinal obstruction, gastrointestinal bleeding, acute pancreatitis and accidental inhalation) within the last 1 month prior to screening.
2. Subjects with medical history that might affect gastrointestinal functions (e.g. inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), short bowel syndrome (SBS), et al.).
3. Subjects who cannot communicate and collaborate normally.

3.3.2 The source and method of population selection.

The study will select the patients who are ≥65 years old and receive long-term hemodialysis treatment in the hemodialysis center, and their clinical manifestations should meet the eligible criteria. We will communicate with the patients about the content and significance of the study, and patients who agree to sign the informed consent will finally be included in the study.

3.3.3 Follow-up.

The schedule of follow-up visits is shown in the flowchart below.

The Study Flowchart

		Screening period	Introduction Period	Treatment Period 1st to 12th Week			Remedial Treatment Period (Only Group B) 13th to 24th Week		
		Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4	Follow-up 5	Follow-up 6	Follow-up 7	Follow-up 8
Follow-up Time Window		-4th to -2nd week	-2nd to 0 week	4th week	8th week	12th week	16th week	20th week	24th week
Eligibility Criteria	Medical History	√							
	Medicine Prescription	√				√			√
	Hemodialysis Parameter	√							
Signature of Informed Consent		√							
Demographic Data		√							
Kt/V			√	√	√	√	√	√	√
URR			√	√	√	√	√	√	√
3-Day Diet Records			√			√			√
Height, Weight, BMI			√			√			√
Upper Arm			√			√			√

Circumference, Upper Arm Skin Fold Thickness								
Grip Strength (Non-Fistula Side)		√			√			√
Abdominal Fat		√			√			√
Body Composition Analysis (Bioresistive Method)		√			√			√
Nutritional Scores (SGA-7)		√			√			√
Life Quality Scores (SF-36)		√			√			√
Gastrointestinal symptoms (GSRS)		√			√			√
Enervation Frailty Scale (EFS)		√			√			√
Pittsburgh Sleep Quality Index		√			√			√
Scr		√	√	√	√	√	√	√
BUN		√	√	√	√	√	√	√
ALB		√	√	√	√	√	√	√
Prealbumin		√	√	√	√	√	√	√
Hb		√	√	√	√	√	√	√
Ca		√	√	√	√	√	√	√
P		√	√	√	√	√	√	√
iPTH		√			√			√
K		√	√	√	√	√	√	√
CO2 Binding Power		√	√	√	√	√	√	√
hs-CRP		√	√	√	√	√	√	√
Ferritin		√	√	√	√	√	√	√
Transferrin Saturation		√	√	√	√	√	√	√
Supply Postbiotics or Placebo		√	√	√	√	√	√	√
Intestinal Flora Determination		√			√			√
Urotoxin Determination		√			√			√

3.3.3.1 Screening Period (-4th to -2nd week before the randomization)

According to previous study, we will screen the CKD-5HD patients who might meet the eligibility criteria. For patients who accept hemodialysis 3 times per week, the first follow-up visit of the screening period should be on the first hemodialysis day of the week (e.g. Monday or Tuesday). Prior to conducting any study procedures, informed consent should be obtained from the patient by the investigator. The patients can provide informed consents within 30 days before the screening procedures.

The following list summarizes the procedures for the screening period:

- We should record demographic information and medical history.
- We should record all medications used by the patient within last 4 weeks prior to the screening.
- We should perform a complete physical examination.
- We should record hemodialysis parameter, including type of vascular access route, current dialysis prescription and dry weight.
- We should record any problem of hemodialysis device, dialyzer and hemodialysis line.
- We should record the most recent URR and spKt/V of patients within last 1 month prior to the screening.
- We should review the eligibility criteria.
- The patients who meet the eligibility criteria after screening period should be divided randomly into the treatment group and control group.

3.3.3.2 Introduction Period (-2nd to 0 week before the randomization)

The second follow-up visit should be on the first hemodialysis day of the week (e.g. Monday or Tuesday). The following list summarizes the procedures for the introduction period:

- We should record the most recent URR and spKt/V of patients within last 1 month prior to the randomization.
- We should record any change of medications after the last follow-up visit.
- We should record the diet of patients.
- We should score the rating scales listed in the flowchart above.
- We should perform the anthropometric measurements listed in the flowchart above.
- We should collect the results of recent routine blood test before dialysis of the patient.
- We should collect the blood and stool samples for urotoxins and intestinal flora baseline measurements.
- The patients will be divided randomly into treatment group and control group. Patients in the treatment group will be given oral intestinal flora metabolites 3 times a day and 1 bag each time. Patients in the control group will be given placebo.
- The therapeutic medications should be supplied.

3.3.3.3 Treatment Period (1st to 12th Week)

Follow-up visits are scheduled at every 4 weeks in the treatment period. The following list summarizes the procedures for the treatment period:

- We should ask patients about any adverse events and changes in medication after last follow-up visit.
- We should record any change of dialysis prescription.
- We should record any problem of hemodialysis device, dialyzer and hemodialysis line.
- We should record the diet of patients in the 12th week.
- We should score the rating scales in the 12th week.
- We should perform the anthropometric measurements in the 12th week.
- We should collect the blood and stool samples for urotoxins and intestinal flora measurements in the 12th week.
- We should collect the results of recent routine blood test before dialysis of the patient in the 4th, 8th and 12th week.
- We should ensure whether the patient has still met the eligibility criteria. If so, supply the medicine at the original dose. If not, remove him/her from the group.

3.3.3.4 Remedial Treatment Period (13th to 24th Week)

Group A end the trial and group B enter the remedial treatment period. Follow-up visits are scheduled at every 4 weeks in the remedial treatment period. The following list summarizes the procedures for the remedial treatment period:

- We should ask patients about any adverse events and changes in medication after last follow-up visit.
- We should record any change of dialysis prescription.
- We should record any problem of hemodialysis device, dialyzer and hemodialysis line.
- We should record the diet of patients in the 24th week.
- We should score the rating scales in the 24th week.
- We should perform the anthropometric measurements in the 24th week.
- We should collect the blood and stool samples for urotoxins and intestinal flora measurements in the 24th week.

- We should collect the results of recent routine blood test before dialysis of the patient in the 16^{th}, 20^{th} and 24^{th} week.
- We should ensure whether the patient has still met the eligibility criteria. If so, supply the medicine at the original dose. If not, remove him/her from the group.

3.3.3.5 Termination.

Patients who complete 24 weeks of follow-up visits are allowed to continue or discontinue their medication supply at the end of the study.

3.4 Outcomes

3.4.1 Primary Outcome Measure:

Outcome Measure	Measure Description	Time Frame
Changes of nutritional status from the baseline to the end of treatment and differences between the intervention group and the placebo group.	Nutritional status of the patient is defined as the results of physical examination and SGA-7 rating scale.	From the baseline to the end of treatment.
Changes of quality of life from the baseline to the end of treatment and differences between the intervention group and the placebo group.	Quality of life of the patient is defined as the scores of gastrointestinal symptom rating scale (GSRS), kidney disease life quality scale (TM-SF36) and enervation frailty scale (EFS).	From the baseline to the end of treatment.

3.4.2 Secondary Outcomes:

Outcome Measure	Measure Description	Time Frame
Changes of enterogenous urotoxin level from baseline to the end of treatment and difference between the intervention and placebo group.	Enterogenous urotoxin level is defined as the serum level of IS, PCS and TMAO. The levels of serum IS, PCS and TMAO will be measured by mass spectrometry.	From the baseline to the end of treatment.

Changes of intestinal flora structure from the baseline to the end of treatment, and the difference between the intervention group and the placebo group.	The structure of intestinal flora will be measured by the V3-V4 region of 16S rDNA with MiSeq PE300 sequencing platform for high-throughput analysis and described by number of the flora.	From the baseline to the end of treatment.
Changes of routine biochemical indicators from the baseline to the end of treatment, and differences between the intervention group and the placebo group.	The routine biochemical indicators include serum creatinine, urea nitrogen, albumin, prealbumin, hemoglobin, serum calcium, blood phosphorus, total parathyroid hormone, serum potassium, carbon dioxide, C-reactive protein, ferritin, transferrin saturation.	From the baseline to the end of treatment.
Changes of dietary intake from the baseline to the end of treatment, and the diffence between the intervention group and the placebo group.	Dietary intakes of the patients will be described by the diet record.	From the baseline to the end of treatment.

3.5 Exposure

Metabolites of intestinal probiotics are those bioactive compounds produced by intestinal bacteria when they consume prebiotics, including short-chain fatty acid, lipolyaccharide, enzyme, exopolysaccharide, cell-free supernatant, inactivated probiotic bacteria or cell fragments (e.g. cell wall fragments, bacterial lysates, et al) and other metabolites (e.g. vitamins, amino acids, et al). Supplying exogenous

intestinal probiotic metabolites is one of the therapies for intestinal flora disorder. It can regulate intestinal flora, improve nutritional status, reduce serum levels of urotoxins and improve quality of life. Patients usually need 8-12 weeks to take the postbiotic product. Here is the introduction of postbiotic product.

The **Postbiotics** used in this study is Yi'en Yuan Honey Fermented Drink, which is made from water, edible brown sugar, and honey as raw materials. It is fermented by a mixture of plant lactic acid bacteria and coagulating Bacillus, and then inactivated at high temperature. The production of the product follows the GB7101 standard, and no live bacteria or related prohibited ingredients are detected in the finished product, which meets the food and beverage standards. According to *NJ-W22070721 Yi'en Yuan Honey Fermented Drink* and *SH-W23040315 Honey enzyme beverage test report*, the product includes carbohydrate 4.0-4.7g/100mL, potassium about 60.9mg/100mL, phosphorus about 2.8mg/mL. According to the concentration shown above, if you consume 150ml of the product a day, you will intake carbohydrate 6.0-9.1g, potassium 90mg, phosphorus 4.2mg, which is far lower than the daily intake of these substances from the diet and can be used for dialysis and diabetic patients. Since its launch in June 2021, no side effects have been reported.

3.6 Sample Size

The study is a randomized control trial. There is no previous related published study so that it’s an exploratory trial. According to the study of other micro ecological preparations, the sample size of each group should be 20. Taking into consideration the dropout rate of 20% by the end of the study, a sample size of 30 per group is planned for this study.

3.7 Data Management

The clinical questionnaire is designed by the doctor whose professional position is or above the associate director. The clinical data is collected and entered by the clinical graduate students participating in the study, while another graduate student verifications the data to ensure the reliability and effectiveness. Each subject is coded by a unique number for subsequent analysis. The clinical data will be encrypted, stored and backed up, and the data analysts should consider of timely updating and integrit. The tests involved in the study are routine clinical tests for MHD patients, and the relevant clinical departments will perform the data entry and storage. The relevant data will be saved by the hemodialysis researchers of the Department of Nephrology, Peking University First Hospital.

The scales involved in the study are commonly used in clinical fields and have been widely used in hemodialysis population. The kidney disease quality of life scale will be completed by the subjects themselves.

3.8 Statistical Analysis

Descriptive statistics for continuous variables will include questionnaire score, anthropometric and biochemical indicators, fecal flora structure and urotoxins level. They will be expressed as mean ± standard deviation and median. Analysis of categorical variables will include frequency and percentage.

In analytical statistics, the distribution type of data will be initially considered when we determine the reference range, and then we will calculate the unilateral lower limit P5. We will compare the distribution of the variables between the normal population and the patients in the two groups with Independent t test, Chi-square test /Fisher exact test, one-way ANOVA and non-parametric analysis. We will analyze the relationship between exposure and outcome with Logistic regression and Cox

regression, including univariate and multivariate regression. In multivariate regression analysis, the selected variables include important general clinical data, the variables clearly associated with the outcome, and the variables analyzed as $P < 0.1$ in univariate regression.

Statistical analyses will be performed using Statistical Package for Social Science (SPSS) for Windows version 23.0 (SPSS Inc. Chicago, IL). Statistical mapping will be performed using GraphPad Prism v.8 (La Jolla, CA, USA) and R 4.1.0 (The R Foundation, Vienna, Austria). Sample size estimation will be performed using PASS 2021 (UT, USA).

$P<0.05$ is considered statistically significant. All confidence intervals are stated at the 95% confidence level.

4. Subject Protection

4.1 Subject Benefit

The study is a crossed randomized control study of metabolites from the gut microbiota and every patient enrolled in the study will receive free use of intestinal probiotic metabolites. The study will provide the relevant test, nutrition and quality of life evaluation free of charge. The examinations in the study are all routine examinations in daily clinical work and do not increase the burden of blood collection for patients. The only invasive test is venous blood sampling for urotoxins measurement, which is carried out according to the routine procedure. The patient needs to collect a stool sample for microflora measurement. The other tests and evaluations are non-invasive.

Regular treatment will not be affected for the patients who do not accept the study. The privacy information of all patients entering the study will be protected and the data will be used in accordance with relevant regulations.

4.2 Subject Risk

During the trial, the treatment group will take 150ml of Yi En Yuan honey yeast drink each day. The product is made of water, edible brown sugar and honey. It's mixed fermented by plant lactic acid bacteria and Bacillus coagulates and sterilized at a high temperature. The product is produced according to GB7101 standard, and there is no live bacteria and prohibited ingredients in the product, which meets the food and beverage standards. According to *NJ-W22070721 Yi En Yuan honey yeast drink* and *SH-W23040315 Honey enzyme beverage test report*, the product includes carbohydrate 4.0-4.7g/100mL, potassium about 60.9mg/100mL, phosphorus about 2.8mg/mL. According to the concentration shown above, if you consume 150ml of the product a day, you will intake carbohydrate 6.0-9.1g, potassium 90mg, phosphorus 4.2mg, which is far lower than the daily intake of these substances from the diet and can be used for dialysis and diabetic patients. Since its launch in June 2021, no side effects have been reported. Patients in the control group will be given 150ml of placebo each day which is made of water and food coloring.

Blood samples of the subjects are taken at the same time as routine clinical tests without additional samples. The risks are the same as those in daily blood drawing tests, including puncture pain, the need for a second puncture when the blood drawing is not successful, and a small amount of subcutaneous bleeding caused by poor compression after blood drawing. Blood drawing will be performed by experienced nurses. The venous blood collection routine will be strictly followed, and after blood

collection we will press effectively to stop bleeding. If the puncture is unsuccessful, we will stop the second puncture and we will try again after 24 hours.

4.3 Confidentiality

This study mainly collects the required data before and after treatment for efficacy observation. Personal datas in the study are confidential. The blood samples are routine clinical laboratory items, and all are daily clinical work. The laboratory department will handle them according to the routine clinical laboratory. Researchers are required to keep the identity of the subjects confidential. Clinical datas and records of the subjects will be kept in a locked filing cabinet and will be accessible only to the investigator. No personal data is disclosed at publication. There are no potential conflicts of interest and no special people involved in this study. The personal data of the subjects in the study will be confidential, and their blood samples will be coded by a unique number rather than their name. No identifying information will be disclosed to anyone other than the researchers, and all researchers are required to keep their identities confidential. In order to ensure that the study is carried out in accordance with the regulations, members of the government management department or the ethics committee can access the personal data of the subjects if necessary, and the process will also strictly follow the principle of confidentiality. No personal information about any subjects will be disclosed when the results of this study are published.

5. Quality Control

5.1 Quality Control of Clinical Research Process

Clinical data collection will be performed by the clinicians. The clinicians participating in this study must have received relevant training and understand

thoroughly about the objectives and procedures of the research. Besides, they should be able to collect high-quality clinical data and biological samples and then pass them to the data analysts and laboratory testers after preliminary collation and store. In the process, they should ensure the rights and interests of the subjects. Each subject will be numbered individually by the clinicians in order to protect their privacy and conduct blind analysis. The data of each subject will be verified by two clinicians to ensure authenticity and reliability.

5.2 Laboratory Process

1）Collection of blood and stool sample: Stool samples will be collected by the subjects themselves, and the fresh stool should be 5ml within 2 hours after defecation. Blood samples will be collected by the nurses, and 5ml of venous blood before dialysis is taken from the subjects on the same day.

2）Packaging and testing of blood and stool samples: After checking the subject number, the specimens will be immediately packaged and stored in the -80℃ refrigerator. The samples will be tested by professional laboratory technicians. Levels of urotoxins will be detected with commercial kits, requiring two multiple holes setting up in each sample with four-parameter Logistic regression method to fit the standard curve with R2≥0.99. Quality control products will be set up for quality control in each test. After preliminary sorting, the test data will be encrypted, stored and backed up for the data analyst.

5.3 Quality Control of Data Management

Part of the data collection will be carried out by trained nurses, and the entry and post-entry verification will be carried out by different personnel. Among the observation indicators involved in the study, the clinical laboratory test results will be uploaded to the database after completion. The electronic medical record system of

the hemodialysis center has a database interface, which can automatically extract and export to the document without manual input process. Statistical principles will be strictly followed to ensure the authenticity of the results, and the results will be interpreted and discussed with the study designers.

5.4 Training

The questionnaires and scores involved in the study, such as quality of life and malnutrition scores, will produce quantitative assessment data, and the specific operation process may affect the results. Therefore, there are specific and complete standardized operation procedures for the operation process of the above scales, and there are special trainings for researchers involved in the evaluation as well.

5.5 Supervision Plan

The person in charge of the study is responsible for the overall coordination of the work among various researchers, planning the work progress of clinicians, laboratory technicians and data analysts, monitoring the quality and efficiency of their work, arranging the transportation and transmission of data and samples, applying for relevant research projects and coordinating budgets and funds. Finally, we will assist data analysts to interpret the research results, and plan the transformation of scientific research results, including patent application, commercial transformation, SCI paper writing and publication.

6. Schedule

The research schedule is shown below.

Time	Work Arrangement
2023-7 to 2023-10	Research project application, testing method debugging, personnel training and coordination
2023-11 to 2024-6	Subject recruitment, clinical data collection and sample testing
2024-7 to 2025-12	Data analysis, result interpretation, SCI paper writing

7. Organization & Management

The organization and management of the study is shown below.

Participating Institutions and Responsible Persons	Responsibility
Chen Yuqing (Peking University First Hospital)	**Overall planning of the project, application for scientific research projects, personnel training and coordination, preservation of biological samples, data collation and analysis, interpretation and discussion of results**
Wan Xin (Nanjing First Hospital)	**Subject recruitment, clinical research, clinical data and sample collection**
Wang Hui (Taiyuan Central Hospital)	**Subject recruitment, clinical research, clinical data and sample collection**
Shen Yulan (Beijing Miyun District Hospital)	**Subject recruitment, clinical research, clinical data and sample collection**

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9. Appendix

9.1 Appendix A. SGA 7 Scale

SGA 7 Scale：6-7 points are classified as very mild malnutrition to good nutrition; 3-5 points are classified as mild to moderate malnutrition; 1-2 points are classified as severe malnutrition.

Subjective Comprehensive Nutrition Assessment Form

Items	Instructions	Score

Change of Weight (Measured in % compared to the weight 6 months earlier) Dry weight 6 months ago:___Kg Dry weight 2 months ago:___Kg Current dry weight:___Kg	+5% is normal 7 points: Body weight stable without change 6 points: There is a recent trend of weight loss >5 to 10% indicates a potential problem 5 points: Weight loss of 5 to 6% 4 points: Weight loss of 7-8% 3 points: Weight loss >10% >10% indicates serious problems 2 points: Weight loss, recent tendency to stabilize or slight change 1 point: Sustained weight loss	
Diet Based on daily intake of the patients and evaluate whether it meets the dietary recommendations	7 points: Normal amount 6 points: Decreased in recent days, but usually moderate 5 points: Continuous reduction, but moderate 4 points: Continue to decrease, sometimes moderate and sometimes not 3 points: Continued to decrease, but not a moderate amount 2 points: Continued to decrease, mostly very little food 1 point: Continued to decrease, but very little food	
Gastrointestinal Symptoms Loss of appetite, nausea, vomiting, diarrhea, etc. The frequency and duration of symptoms.	7 points: Asymptomatic 6 points: Symptoms are rare 5 points: One or more symptoms occur, but not daily 4 points: Appear more than one per day 3 points: Most symptoms appear daily 2 points: A day with almost all symptoms 1 point: Daily occurrence of all symptoms	
Ability of Daily Living (Compared to 6 months ago) Work, family, social	7 points: General condition 6 points: Worse than average, but not related to nutritional status 3-4-5 points: Worse than average, but related to nutritional status 2 points: Bedridden or wheelchair, related to nutritional status but can perform simple activities 1 point: bedridden, related to nutritional status but unable to perform simple activities	
Metabolic Stress	7-6 points: Only ESRD or (and) well-controlled DM 4-5 points: DM with infection/fever/peritonitis/poor control 1-2-3 points: Serious diseases such as ulcerative colitis with diarrhea	
Physical Examination Measure muscle layer and fat layer	1 point (sunken) - 7 points(full) fat: eyelids/triceps/biceps 1 point (bone protrusion) - 7 points (bone not easily seen) Muscles: temples/scapula/clavicle/ribs/jaws and interphalanges of palms/quadriceps/knees 1 point (severe edema) -7 points (no edema) : Edema of fibula or ankle	
Total	Do not average; Overall nutritional status and clinical experience are evaluated to range from 1 to 7 points.	

The result is shown as follows:

Items		Score
Change of Weight		
Diet		
Gastrointestinal Symptoms		
Ability of Daily Living		
Metabolic Stress		
Physical Examination	**Fat**	
	Muscle	
	Edema	
Total		

Physical Examination of Fat and Muscle:

Fat: Eyelids, triceps, biceps. 1 point is classified as sunken; 7 points are classified as full.

Muscle: temples, scapula, clavicle, ribs, jaws and interphalanges of palms, quadriceps, and knees. 1 point is classified as bone protrusion; 7 points are classified as bone not easy seen.

Researchers should give an overall score (from 1 to 7) refer to the combination of fat and muscle assessment and the final average should be rounded.

Change of Weight of 6 Months:

Body weight stable without change: 7 points.

Weight loss less than 3%: 6 points.

Weight loss of 3-5%: 5 points.

Weight loss of 5-7%: 4 points.

Weight loss of 7-10%: 3 points.

Weight loss of 10-15%: 2 points.

Weight loss more than 15%: 1 point.

9.2 Appendix B. Kidney Disease Quality of Life Scale（TM-SF36）

1. Generally speaking, do you think your health status is:				
Superb	Very good	Good	Normal	Terrible
1	2	3	4	5

The following items are about the activities you may do during the day. Does your current state of health limit your ability to do these activities? If so, how much?			
	Yes, limited a lot.	Yes, limited a little.	No, not limited at all.
2. Moderate activities, such as moving the table, sweeping the floor, playing badminton or swimming	1	2	3
3. Climbing a few flights of stairs	1	2	3

During the past 4 weeks, did you have any of the following problems while working or performing daily activities due to your health condition?		
	Yes	No
4. Doing less than wish	1	2
5. Limited ability in working and daily activities.	1	2

In the past 4 weeks, did you have any of the following problems while working or performing daily activities due to emotional condition, such as feeling depressed or anxious?		
	Yes	No
6. Doing less than wish	1	2
7. Not as carefully as the past in daily work or activities.	1	2

8. In the past 4 weeks, how much did the pain interfere with your normal work, including working outdoors and housework?				
Not at all	A little	Moderate	Severe	Extreme

1	2	3	4	5	
These questions are related to your feelings and life over the past 4 weeks. For each question, please give an answer.					
	All time	Most of time	Many time	Sometime	Seldom	Never
9. Do you feel peaceful?	1	2	3	4	5	6
10. Do you feel energetic?	1	2	3	4	5	6
11. Do you feel depressed?	1	2	3	4	5	6
12. During the past 4 weeks, how many times did your physical health or emotional problems interfere with your social activities (e.g. visiting friends)?					
All time	Most of time	Sometime	Seldom	Never	
1	2	3	4	5	
About your kidney disease, how much are these following statements true or untrue to you?					
	Definitely true	Mostly true	Don't know	Mostly untrue	Definitely untrue
13. My kidney disease interferes too much with my life.	1	2	3	4	5
14. I waste too much time to coping with my kidney.	1	2	3	4	5
15. I'm depressed about coping with my kidney.	1	2	3	4	5
16. I think I'm the burden of my family.	1	2	3	4	5
During the past 4 weeks, how much were you troubled by any of the following?					
	No trouble	Little trouble	Moderate	Heavy trouble	Extreme trouble
17. Muscle soreness					
18. Chest pain					
19. Cramp					
20. Cutaneous seizure					
21. Dry skin					
22. Tachypnea					
23. Giddy					
24. No appetite					
25. Fatigued					
26. Numbness of hands and feet					
27. Vomiting or stomachache					
28. Is there any problem with the vascular site where you inserted the catheter?					
Some people's daily lives are troubled by kidney disease, others are not. How much are you troubled by the kidney disease in the following ways?					
	No trouble	Little trouble	Moderate	Heavy trouble	Extreme trouble
29. Limit of drink					
30. Limit of diet					
31. Ability of chores					
32. Ability of travel					
33. Dependence to doctors and other medical staff					
34. Depression caused by kidney disease					

35. Sexual life					
36. Personal instrument					

9.3 Appendix C. Gastrointestinal Symptom Rating Scale (GSRS)

Did you have any of the following symptoms in the past week?		Score						
		No	Mild	Sometimes	Moderate	Obvious discomfort	Quite serious	Extremely serious
1	Epigastric pain	1	2	3	4	5	6	7
2	Chest pain	1	2	3	4	5	6	7
3	Acid regurgitation	1	2	3	4	5	6	7
4	Hunger pain	1	2	3	4	5	6	7
5	Nausea	1	2	3	4	5	6	7
6	Borborygmus	1	2	3	4	5	6	7
7	Abdominal bloating	1	2	3	4	5	6	7
8	Sore throat	1	2	3	4	5	6	7
9	Halitosis	1	2	3	4	5	6	7
10	Smelly urine	1	2	3	4	5	6	7
11	Constipation	1	2	3	4	5	6	7
12	Diarrhea	1	2	3	4	5	6	7
13	Loose stool	1	2	3	4	5	6	7
14	Dry stool	1	2	3	4	5	6	7
15	Defecate immediately if you want to	1	2	3	4	5	6	7
16	Tenesmus	1	2	3	4	5	6	7
Total								

9.4 Appendix D. Enervation Frailty Scale（EFS）

				Score:_____ /17
		0 Point	1 Point	2 Points
Clock test (cognition)	1. Think of this pre-drawn circle as a clock. 2. Write the number down on the dial in the correct position. 3. Put the pointer in the "eleven ten" position.	No mistake	Mild mistake	Other mistake
General health state	How many times did you hospitalize in the past year?	0	1-2	>2
	How would you describe your overall health?	Very good & Good	Normal	Terrible
Functional independence	How many of the following activities do you need help? (Cooking, shopping, transportation, phone calls, housework, laundry, financial management, medication)	0-1	2-4	5-8
Social Support	When you need help, do you think there will be someone willing to help and able to meet your needs?	Often	Sometimes	Never
Medication	Do you take 5 or more pills a day?	No	Yes	
	Would you forget to take medicine?	No	Yes	
Nourishment	Did you lost any weight recently, such as your clothes becoming looser?	No	Yes	
Emotion	Do you often feel depressed?	No	Yes	
	Do you have trouble in controlling urinating when you don't want to urinate?	No	Yes	
Somatic function	Sit in the chair with your back and arms relaxed. Then, when I say "go," stand up and walk at a safe and comfortable pace to the mark on the floor (about 3 meters away), then return to your chair and sit down.	0-10s	11-20s	More than 20s, or unwilling to act, or need assistance to act

Evaluation: 0-5 points are defined as not weak; 6-7 points are defined as vulnerable; 8-9 points are defined as mild weakness; 10-11 points are defined as moderate weakness; more than 11 points are defined as severe weakness.

9.5 Appendix E. Pittsburgh Sleep Quality Index

1. What time do you usually go to bed in the past month?
2. How long did it usually take you to fall asleep every night for the past month? Minutes
3. What time did you usually get up every morning for the past month?
4. How much sleep did you actually get every night in the past month? Minutes

5. In the past month, did you often have trouble in sleeping because of the following statements?				
	Not in the past month	Less than one night a week on average	One or two nights a week on average	Three or more than three nights a week on average
A. Cannot sleep within 30mins				
B. Waking up in midnight or wake up early				
C. Getting up at night to the toilet				
D. Uncomfortable breathing				
E. Coughing or snoring loudly				
F. Feeling cold				
G. Feeling hot				
H. Bad dreams				
I. Pain				
J. Other: Please describe				

6. How do you think of your total sleep quality over the past month?			
Very good	Good	Not good	Terrible

7. In the past month, did you often have to take medication (including prescription or from an outside pharmacy) to fall asleep?	
Not in the past month ()	Less than one night a week on average ()
One or two nights a week on average ()	Three or more than three nights a week on average ()

8. In the past month, did you have trouble in staying awake while driving, eating, or participating in social activities?	
Not in the past month ()	Less than one night a week on average ()
One or two nights a week on average ()	Three or more than three nights a week on average ()

9. In the past month, did you have any difficulty in completing tasks?			
Not difficult	Mild difficult	Quite difficult	Very difficult

10. Do you share a bed or have a roommate?	
No. ()	Partner or roommate is in another room ()
Partner or roommate is in the same room but does not share a bed ()	Partner or roommate share a bed ()

11. If you share a bed or have a roommate, please ask him/her if any of the following happened to you in the past month.				
	Not in the past month	Less than one night a week on average	One or two nights a week on average	Three or more than three nights a week on average
A. Do you snore when you sleep?				
B. Do you have long pauses between your breaths during your sleep?				
C. Do your legs twitch or have spasms during your sleep?				
D. Are you disoriented or confused during your sleep?				
E. Have you had any other trouble during your sleep? Please describe it.				