Research Protocol

HORUZ study

Investigating the Hemodynamic profile Of chronic Renal patients by Use of bioimpedance

Melanie K. Schoutteten

Willemijn Groenendaal

Chris van Hoof

Jeroen P. Kooman

Jacques Peeters

Christophe JP. Smeets

Pieter M. Vandervoort

NCT number: not yet available

3th December 2019

Coordinating investigator: M.K. Schoutteten *MD*

Principal investigator: P.M. Vandervoort *MD PhD*

Principal local investigator per site:

Name Site 1: **Ziekenhuis Oost-Limburg, Schiepse Bos 6, 3600 Genk, België**

- Name local researcher: Melanie Schoutteten
- E-mail local researcher: melanie.schoutteten@uhasselt.be

Coordinates contact person:

- Name site: Ziekenhuis Oost-Limburg
- Name contact person: Dr. Melanie Schoutteten
- E-mail contact person: melanie.schoutteten@uhasselt.be
- Telephone contact person: 0032 (0) 89 21 20 59

Sponsors (in Dutch: verrichter/opdrachtgever):

Ziekenhuis Oost-Limburg, Schiepse Bos 6, 3600 Genk, België

Laboratory sites:

Ziekenhuis Oost-Limburg, Schiepse Bos 6, 3600 Genk, België

PROTOCOL SIGNATURE SHEET

Name	**Signature**	**Date**
Coordinating Investigator ***M.K. Schoutteten MD***	Removed on request of Clinicaltrails.gov	25.10.2019
Principal Investigator ***P.M. Vandervoort MD PhD***	Removed on request of Clinicaltrails.gov	25.10.2019

TABLE OF CONTENTS

LIST OF ABBRIVIATIONS AND RELEVANT DEFINITIONS

BCM	body composition monitor
BIS	bioimpedance spectroscopy
BP	blood pressure
CKD	chronic kidney disease
CRP	C-reactive protein
CrCl	creatinine clearance
CrUrCl	mixed creatinine and urea clearance
CV	cardiovascular
DSMB	data safety monitoring board
ECG	electrocardiography
ECV	extracellular volume
ECW	extracellular water
EDD	end diastolic diameter
eGFR	estimated glomerular filtration rate
ESRD	end stage renal disease
FO	fluid overload
FS	fluid status
ICD	implantable cardiac defibrillator
ICV	intracellular volume
ICW	intracellular water
IVS	interventricular septum
LTI	lean tissue index
LVEF	left ventricle ejection fraction
LVH	left ventricular hypertrophy
LVMI	left ventricle mass index

METC	medical research ethics committee, in Dutch: medisch ethische toetsing commissie
MRI	magnetic resonance imaging
OH	overhydration
PCR	urine protein-to-creatinine ratio
PM	pacemaker
PWT	posterior wall tickness
RCT	randomized controlled trial
RRF	residual renal function
RWT	relative wall thickness
(S)AE	(serious) adverse event
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study, but does not commission it, is not regarded as the sponsor, but referred to as a subsidizing party.
SF36	short form 36 questionnaire
SUSAR	suspected unexpected serious adverse reaction
TBW	total body water
Tx	transplantation

SUMMARY

Rationale: Preservation of residual renal function is independently associated with reduced mortality and improved outcomes in patients with end stage renal disease. Fluid status and blood pressure influence the rate of kidney function decline early in the course of chronic kidney disease (CKD). The technique of bioimpedance spectroscopy (BIS) can offer valuable information on fluid dynamics and body composition, in addition to standard care.

Objectives: This study aims to investigate the role of thoracic and whole body BIS in the hemodynamic profile of patients with CKD. The primary objective is to associate the BIS-determined fluid status [fluid overload (FO) versus non-FO] with kidney function decline in patients with CKD stage 3 (CKD-EPI 59-30ml/min), 4 (CKD-EPI 29-15ml/min) and 5 (CKD-EPI < 15 ml/min). The secondary objective is to identify the clinical factors associated with FO during a longitudinal follow up. Secondary outcomes of interest are the central volume status (measured by echocardiography and thoracic BIS in combination with photoplethysmography), the cardiac systolic and diastolic function, CV events, hospitalizations, inflammatory- and nutrition status, and quality of life, between CKD stages 3, 4 and 5 and compare them to an age- and sex- matched healthy control group.

Study design: An non-randomized intervention study with a longitudinal follow up of the cohort and some cross-sectional analysis.

Study population: Prevalent CKD patients stage 3 - 5 not on dialysis and an age- and sex- matched healthy control group, will be included during a period of 18 months and prospectively followed up over a period of 12 months.

Intervention: During each study visit BIS- and blood pressure measurements will be performed, and blood- and urine samples will be taken in each subject.

Main study parameters/endpoints:

- Whole body fluid status, measured by whole body BIS
- Central volume status, measured by thoracic BIS, photoplethysmography and echocardiography
- Blood pressure, measured by automatic oscillometric device
- Determinants of BIS signal in CKD population: gender, comorbidities
- Rate of kidney function decline, determined by eGFR CKD-EPI, mixed urea and creatinine clearance, and urine volume measured by 24h urine collection

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

- BIS and blood pressure measurements will be achieved by non-invasive techniques which pose a minimal burden to the patient.
- Blood sampling will as much as possible coincide with regular blood samplings for clinical purposes. The risk of venipuncture-related complications is considered moderate when performed in the non-dominant arm, as this can influence negatively on a future vascular access.
- All subjects undergo echocardiography. Analysis of the echocardiography will be done during follow-up period. Aberrant results will be discussed with an expert.
- All study-related costs will not be charged to the patient.

INTRODUCTION AND RATIONALE

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health.[1] Classification of CKD is structured according to the estimated glomerular filtration rate of creatinine (eGFR) and albuminuria (Figure 1). CKD is a major health problem and continues to rise globally. Prevalence of CKD is estimated at 11-13% of the adult population, of which 0.1% results in end stage renal disease (ESRD, defined as eGFR < 15ml/min or the need for renal replacement therapy).[2,3] As well in developed as in developing countries, aging and the higher rate of cardiovascular (CV) events in the general population explains the increase of CKD.[2] Not only prevalence, but also mortality in CKD population is mainly determined by CV comorbidities.[4,5] Besides the Framingham classics, novel CV risk factors such as fluid status (FS), malnutrition, inflammation and impaired kidney function itself have gained importance.[4,6,7]

Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

GFR categories (ml/min/ 1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Figure 1 Classification of chronic kidney disease according to glomerular filtration rate and albuminura. Colors represent the cardiovascular prognosis. Green: low risk; yellow: moderately increased risk; orange: high risk; red: very high risk.

As glomerular function, and salt- and water excretion decline, extracellular fluid expansion develops, causing hypervolemia.[8] **Fluid overload** (FO) in Asian populations with CKD stages 3-5 is cause and consequence of renocardiac complications,[6] leads to progression of CKD,[9] and is associated with increased risk for all-cause mortality or CV morbidity.[10]

Hypervolemia and hypertension in CKD population represent the hemodynamic triggers to formation of **left ventricular hypertrophy** (LVH), respectively as increased preload and increased afterload.[11] Prevalence of LVH is estimated at 45-79% in the predialysis group,[12-15] and rises to 75-85% after start of HD.[13,16] LVH leads to left ventricle stiffness and this results in diastolic heart failure.[17] LVH is independently associated with increased mortality and CV events.[18-20]

FO is one of the multifactorial pathophysiological causes of chronic systemic **inflammation** in the CKD population. Inflammation, assessed by C-reactive protein, serum-albumin or ferritin levels, and **malnutrition** accelerate the generalized atherosclerosis in advanced CKD, leading to CV complications and progression of kidney function decline.[21-23]

Kidney function decline is associated with an exponential increase in risk of death, left ventricle mass index, blood pressure (BP), CV events and hospitalization, and reduced removal of uremic toxins, independent from classical risk factors.[4,7,24]

Based on the above mentioned associations between risk factors and outcome in the CKD population, the question rises what specific hemodynamic profile (including FS and BP) is associated with a severe CV comorbidity cascade leading towards more CV events, hospitalizations, rapid decline of kidney function and acceleration of the initiation of renal replacement therapy.

Assessment of the hemodynamic profile, cardiac- and kidney function in predialysis patients

During progression to ESRD, accumulation of fluid occurs silently. Clinical assessment of FS is not straightforward in CKD patients. While prevalence of overhydration in CKD stages 3 - 5 is estimated at 50%,[6,25,26] 20% of these patients is non-edematous at clinical examination.[6] The gold standard to measure fluid distribution in the human body is dilution of deuterium and sodium bromide solution for extracellular water (ECW) and total body potassium for intracellular water (ICW). However, these techniques are invasive and not applicable in routine clinical practice. **Bioimpedance spectroscopy** (BIS) is a noninvasive and easy-to-use technology in fluid assessment. Some BIS instruments have been validated in hemodialysis patients against dilution methods.[27-30] BIS provides the possibility to estimate total body water (TBW), extracellular volume (ECV), intracellular volume (ICV), fat-free mass, fat mass and muscle mass.[31,32] Also, blood volumes can be accurately estimated by BIS and can optimize BP treatment.[33]

In contrast to the hemodialysis population, the clinical use of BIS in fluid management of non-dialysis CKD stages 3 - 5 is less frequently implemented. Observational research has shown that BIS identifies FO in 20% of non-edematous patients with CKD stages 3 - 5, and showed impairment of body composition in absence of overt signs of malnutrition.[6,25] BIS-derived parameters as FO or lean tissue mass are associated with more rapid decline in residual renal function (RRF), higher CV morbidity and mortality compared to euvolemic CKD-patients.[9,10,26]

The combination of electrocardiography (ECG) and echocardiography is the easiest and cheapest reliable method to evaluate LVH. However, the gold standard is magnetic resonance imaging (MRI), this is not widely used because of individual MRI tolerance and costs.

Clinical evaluation of kidney function decline is currently performed by longitudinal follow up of serum creatinine concentration and eGFR of creatinine (expressed by the eGFR CKD-EPI equitation), and albuminuria.[1,34] However, due to the variability of creatinine production and tubular secretion, eGFR

or creatinine is often precluded from clinical trials. Creatinine clearance [creatinine clearance (*CrCl*) = (urine creatinine concentration x urine volume) / serum creatinine concentration] or mixed creatinine and urea clearance (CrUrCl) improves accuracy.[35]

Hemodynamic management of CKD progression and CV morbidities in predialysis patients

Prevention of progression to ESRD and CV complications in predialysis patients is currently addressed by targeting the classical risk factors (BP and glycemic control, salt intake and dietary advice, smoking cessation, albuminuria, physical activity). Management of novel CV risk factors like **FS** is not incorporated in the KDIGO CKD guidelines.[1] Furthermore, the recommendation to keep **BP** in CKD patients ≤ 140 mmHg systolic and ≤ 90 mmHg diastolic because of reduced risk of CV mortality and CV events, is based on office measurements.[1,36] Home-measured BP is more strongly associated with LVH and is a stronger predictor of CV risk than office BP.[37-40] Also, there is a lack of evidence for the optimal medical management of **heart failure** in ESRD population.[1] The effect of BP control on LVH in CKD patients has not been clearly evaluated yet.[36,41]

Preservation of **RRF** in hemodialysis patients is associated with prolonged survival and reduction in CV morbidities. Hence, we should move forward in our attempts to preserve renal function and start with innovative interventions in the predialysis period.

Hypothesis

We hypothesize that FO assessed by BIS is associated with a more rapid decline in RRF and that the follow up of the thoracic BIS signal is an equivalent predictor for cardiac function compared to echocardiography. Eventually we hope that our intervention might lead to a better hemodynamic

management, deceleration of kidney function decline and reduction of CV morbidity and mortality in CKD population, although this is beyond the scope of this research study.

In order to investigate this hypothesis, we want to perform the HORUZ study. We are interested in the fluid status, BP and thoracic BIS signal of patients with CKD 3 – 5, not on dialysis. We aim to provide more evidence to further support the frequent use of BIS in diagnostic guidelines of patients with CKD.

OBJECTIVES

The primary objective of the HORUZ study is to associate fluid status with kidney function decline in patients with CKD stage 3 (CKD-EPI eGFR 60-30ml/min), 4 (30-15ml/min) and 5 (< 15 ml/min) and compare them to an age- and sex- matched healthy control group. Primary outcome is the mean eGFR decline which will be compared between the FO-group and the non-FO-group.

The secondary objective is to identify the clinical factors associated with FO during a longitudinal follow up. Secondary outcomes of interest are the central volume status (measured by echocardiography and thoracic BIS in combination with photoplethysmography), the cardiac systolic and diastolic function, CV events, hospitalizations, inflammatory- and nutrition status, and quality of life, between CKD stages 3, 4 and 5 and compare them to an age- and sex- matched healthy control group.

STUDY DESIGN

The HORUZ study is a non-randomized trial with intervention of regular BIS- and BP measurements, blood- and urine sampling. Prevalent patients with CKD stages 3, 4 and 5 and an age- and sex- matched healthy control group will be included. Inclusion period will last 18 months and CKD-patients will be followed up for 12 months.

STUDY POPULATION

1. **Population**

206 patients with CKD stages 3-5 will be included from the outpatient clinic of Ziekenhuis Zuid-Oost Limburg in Genk (Belgium).

A healthy control group for the cross-sectional part will be recruited through visitors and/or personnel of Ziekenhuis Oost-Limburg in Genk (Belgium).

2. **Inclusion criteria**

In order to be eligible to participate in this study, a subject must meet the following criteria:

- Prevalent non-dialysis patients with eGFR < 60 ml/min (for CKD subjects)
- Age > 18 years
- Signed informed consent

3. **Exclusion criteria**

A subject who meets any of the following criteria will be excluded from participation in this study:

- Acute kidney injury
- eGFR < 20ml/min directly post-nephrectomy
- Clinical conditions affecting BIS measurements: limb amputation, impaired skin integrity, brain stimulator, pacemaker with low threshold or unipolar pacemaker.
- Clinical conditions affecting body composition: liver cirrhosis, active infectious disease or any acute CV event (defined as cerebrovascular event, myocard infarction, decompensatio cordis) during the 3 months before screening for inclusion
- Pregnancy

- Not able to sign informed consent

4. Sample size calculation

The primary outcome parameter is the mean eGFR decline which will be compared between the FO-group and the non-FO-group. We hypothesize that the mean difference between eGFR between these two groups will be 2 µmol/ml/year. Because the study of Levin et al. does not report a SD, we take the SD of the MDRD study[1]: 4.7 µmol/ml/year[1,42,43]. In order to show an alpha of 0.05 and a power of 0.8, at least 86 patients would be needed per group. In order to correct for drop-outs, at least 103 patients per group have to be included.

METHODS

1. Study parameters/endpoints

1.1 Demographic and clinical data

The prevalent CKD stage 3 – 5 population will be screened by the researcher. After verification of the in- and exclusion criteria, eligible patients will be invited to participate the HORUZ study, with approval of their treating nephrologist. After signing the informed consent, data at baseline and during follow-up visits will be collected on paper clinical report forms (Table 1). Hypertension is defined as an office BP ≥ 140/90 mmHg or home BP ≥ 130/80 mmHg, or current medication for hypertension.[1] Diabetes mellitus will be assumed to be present in patients who report use of insulin and/or oral hypoglycemic agents. CV diseases include coronary artery disease, as documented by coronary angiography or a history of myocardial infarction, congestive heart failure, cerebrovascular accident, or known peripheral vascular disease. Fluid assessments, including BIS measurements, will be recorded on the HORUZ registration form (Appendix).

The healthy controls included for the cross-sectional analysis will only be followed according to visit 0 and 1.

Table 1 Schedule of the main data to collect in CKD subjects.

	Baseline Visit 0	Month 1 Visit 1	Month 3 Visit 2	Month 6 Visit 3	Month 9 Visit 4	Month 12/ end of study Visit 5
Consent	X					
Demographics: Age (y) Gender Height (cm) Smoking status Etiology CKD Hypertension Diabetes CV disease Tx list	X					
$eGFR_{creat}$	x	x	x	x	x	x
CrUrCl		x	x	x	x	x
24h urine		x	x	x	x	x

BP	x		x	x	x	x
ECG	x		x	x	x	x
Echo cor		x				x
CV events		x	x	x	x	x
Hospitalization		x	x	x	x	x
Medication	x		x	x	x	x
Whole body BIS	x		x	x	x	x
Thoracic BIS	x		x	x	x	x
SF36	x					x
Adverse event		x	x	x	x	x

Abbreviations: BIS, bioimpedance spectroscopy; BP, blood pressure; CKD, chronic kidney failure; Creat, creatinine; CrUrCl, mixed creatinine and urea clearance; CV, cardiovascular; ECG, electrocardiography; M, month; SF36, short form 36 questionnaire; Tx, transplantation.

1.2 Primary endpoint

The association between fluid status and kidney function decline in patients with CKD stage 3 (CKD-EPI 60-30ml/min), 4 (CKD-EPI 30-15ml/min) and 5 (CKD-EPI < 15 ml/min) is the primary objective of the HORUZ study.

The primary endpoint is the mean eGFR decline which will be compared between the FO-group and the non-FO-group. BIS-measured FO will be expressed as overhydration (OH) normalized to ECW and noted as a percentage of ECW (OH/ECW).[6,44] FO is defined as an OH/ECW ≥ 7% (or an absolute OH ≥ 1.1 L).[45]

Rate of kidney function decline will be assessed by the slope in $eGFR_{creat}$ CKD-EPI (see formula below), defined as the regression coefficient between $eGFR_{creat}$ and time, in units of mL/min/1.73m^2 per year. At least 3 $eGFR_{creat}$ values are required to evaluate $eGFR_{creat}$ slope. All $eGFR_{creat}$ CKD-EPI will be uniformly calculated in the laboratory. $eGFR_{creat}$ decline is assessed by changes in eGFR over time. Rapid $eGFR_{creat}$ decline is defined as the quartile with the steepest eGFR slope ($eGFR_{creat}$ decline > 3mL/min/1.73m^2 per year).[9]

$$eGFR_{creat} = 141 \times \min(S_{creat}/\kappa, 1)^{\alpha} \times \max(S_{creat}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]}$$

S_{creat} *(standardized serum creatinine) = mg/dL*
κ *= 0.7 (females) or 0.9 (males)*
α *= -0.329 (females) or -0.411 (males)*
min = indicates the minimum of S_{creat}/κ *or 1*
max = indicates the maximum of S_{creat}/κ *or 1*
age = years

Average CrUrCl will be determined by creatinine and urea measurements in blood- and urine samples (Table 2).

Residual urine output (mL per 24h) will be collected every study visit and analyzed on volume, urea, creatinine, sodium, osmolarity and albumin/creatinine ratio (ACR) (Table 2).

Time to renal replacement therapy will be recorded.

1.3 Secondary endpoints

The secondary objective is to identify the clinical factors associated with FO during a longitudinal follow up. Secondary outcomes of interest are the central volume status (measured by echocardiography and thoracic BIS in combination with photoplethysmography), BP, the cardiac systolic and diastolic function, CV events, hospitalizations, inflammatory- and nutrition status, and quality of life, between CKD stages 3, 4 and 5.

1. *Cardiac function:* Electrocardiography and echocardiography (see point 3.3) will be scheduled at baseline, and at 12 months or at end of study visit.
2. *Thoracic BIS values:* a wearable device from Imec Eindhoven will be used to measure thoracic BIS values in combination with photoplethysmography and cardiopulmonary index.
3. *Blood pressure:* patients will be asked to take BP measurements at home three times a week twice daily with an automatic BP monitor. Mean arterial pressure corrected for heart

frequency will be calculated. BP measurements can be transmitted to the researcher by use of the local Dharma platform.

4. *CV events:* CV events defined as hospitalization for coronary disease, heart failure, stroke or peripheral artery disease will be recorded during follow-up.
5. *Hospitalization rate – and indication:* hospitalization rate and – indication will be listed.
6. *Inflammatory- and nutrition status:* CRP, ferritin and albumin will be determined every visit (Table 2). Fat tissue mass and lean tissue mass will be analyzed by BIS measurements.
7. *Quality of Life assessment:* SF36 questionnaire will be filled in at baseline and end of study visit.

Table 2 Overview of parameters evaluating kidney function, renal metabolism and inflammation in HORUZ study.

Assay	Material	Standard care	Frequency
Residual renal function			
Urea	Plasma/serum	yes	Every study visit
Creatinine			
Metabolism and electrolytes			
Hemoglobin	Plasma/serum	yes	Every study visit
Sodium			
Potassium			
Bicarbonate			
Phosphate			
Calcium			
NT-proBNP		No	Every study visit
Inflammation			
C-reactive protein	Plasma/serum	yes	Every study visit
Leucocytes			
Albumin			
Ferritin			
24 urine collection			
Quantity	Urine	No	Every study visit
Creatinine			
Urea			
Sodium			
Albumin			
Protein			

2. Randomization

Not applicable.

3. Study procedures and interventions

After inclusion during the baseline visit, a research visit will be scheduled every 3 months until end of study or withdrawal. Study visits will be scheduled as much as possible before the regular visit to the treating nephrologist. During this period of follow up, all participants will undergo the following procedures to determine the predefined research parameters (Table 1):

At the first visit, patient's medical file will be checked for baseline demographic information, comorbidities, risk factors and medication use. Patients will be asked about complains or symptoms related to fluid homeostasis (weight gain, shortness of breath, thoracic pressure, palpitations, muscle cramps, dizziness, fatigue, thirst, obstipation). Physical signs of fluid imbalance (subcutaneous edema) will be evaluated by physical examination. Subjects will be requested to collect 24h urine sample and take a blood sample to determine the mixed urea and creatinine clearance. BP, ECG and echocardiography will be performed in all subjects. BIS will be performed by the reference and wearable device. Patients will be asked to fulfill the SF36 questionnaire.

During subsequent visits, anamnesis, physical examination, blood- and urine samples, BP and BIS measurements will be taken from all subjects.

At 3 and 12 months of follow up, additional evaluation of cardiac function and quality of life will be performed.

3.1 BP measurements

All subjects will be asked to measure BP three times a week, twice a day (morning and evening before sleeping). BP will be measured by an automatically oscillometer. The researcher will evaluate the BP each study visit. A mean value of morning and evening measurements will be calculated.

3.2 BIS measurements

Multifrequency BIS measurements will be performed using a commercially available reference device [i.e. Fresenius Medical Care, Body Composition Monitor® (BCM)] (Figure 2), and a research device from Imec Eindhoven® (Figure 3).

Figure 2 Body composition monitor

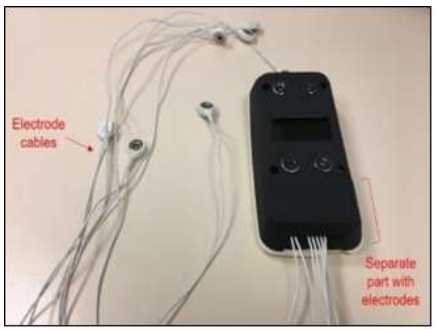

Figure 3 Wearable Imec BIS device

Measurements are non-invasive. For every BIS measurement, the patient is asked to lie down in the most comfortable position at around 20/30° supine position with at least 5 minutes of equilibration time before measurements starts. For the reference device, a standardized electrode configuration will be used: two electrodes attached on the foot and two on the hand, ipsilateral. If for one or another reason, it is not possible to attach it on the right side of the body, the left side will be used. No other measurements will be taken simultaneously (i.e. ECG). The applied micro current is alternating between 5 and 1000 kHz.

Next, the wearable research device is attached to the thorax using a total of 9 electrodes, 4 electrodes serve for BIS measurement, 1 bias, 2 for ECG, and 2 other electrodes are necessary to attach the research device to the patient and keep it in a stable position. The five leads are placed in a standardized manner as shown in Figure 4. Each measurement takes 20 minutes.

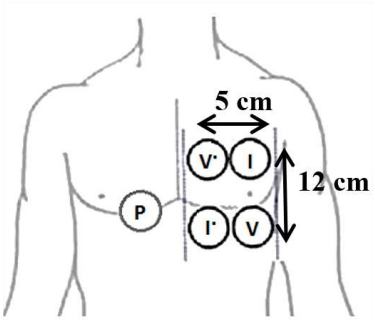

Figure 4 *Thoracic electrode configuration of the research device, BIS measurement.*

I, current; V, voltage

3.3 Cardiac function

At baseline (Visit 0), Visit 2 and Visit 5 of the study, ECG will be performed. At baseline and end of the study, an echocardiography will be planned to evaluate cardiac function. Echocardiography will be performed by professional echocardiographists according to a standardized protocol (in Dutch):

1. *Parasternale lange as (PLAX)*
2. *Parasternale korte as (PSAX)*
3. *Apicale 4 kamer (A4C) window + idem met nauwe sector width voor offline strain (+ color)*
4. *Apicale 2 kamer (A2C) window + idem met nauwe sector width voor offline strain (+ color)*
5. *Apicale 3 kamer (A3C) window + idem met nauwe sector width voor offline strain (+ color)*
6. *Graag ook in A4C licht uitgedraaid LA beeld met nauwe sector width (voor offline LA strain)*
7. *Graag RV modified view nauwe sector width*
8. *Graag 3D TTE-beeld (bij voorkeur 4 loop beat, zo belangrijke stitching high volume rate)*
9. *Graag 3D-beeld RV (bij voorkeur 4 loop beat, zo belangrijke stitching high volume rate)*
10. *Subcostaal (met VCI view)*
11. *Pulsed wave at opening MV leaflets*
12. *Pulsed wave pulmonaal vene*
13. *TDI septale mitraal annnulus*
14. *TDI laterale mitraal annulus*
15. *Pulsed wave LVOT*
16. *Continuous wave AV*
17. *TAPSE RV*
18. *TDI met PW op Tricuspiedannulus (voor RV S')*
19. *So moderate to servere MI : downshift baseline voor offline PISA*
20. *RVSP*

Er moeten geen online metingen uitgevoerd worden.

Measurements will be performed by a specialized nurse or the researcher. The parameters will be analyzed by the researcher to minimize inter-observer variability. The following echocardiographic parameters will be ascertained: left ventricle mass index (LVMI = LVM/m^2), left ventricular hypertrophy (LVH), relative wall thickness (RWT), left ventricle ejection fraction (LVEF), left ventricle geometry, and diastolic dysfunction.[12,17]

LVM will be calculated via the area-length method [specific gravity and LV muscle volume (epicardial volume minus chamber volume, or endocardial volume)]:

$$\text{LVM (g)} = \rho\,[\tfrac{5}{6}A_1(L+t) - (\tfrac{5}{6}A_2\,L)]$$

$$\rho = specific\ gravity\ of\ muscle = 1.05 g/mL$$

$$A_1 = epicardial\ area\ at\ end\ diastole\ (\text{cm}^2)$$

$$A_2 = endocardial\ area\ at\ end\ diastole\ (\text{cm}^2)$$

$$L = ventricle\ length\ at\ end\ diastole\ (cm)$$

$$t = average\ wall\ thickness\ (cm)$$

LVH will be defined according to the Cornell Criteria: normal (0.50g/m^2 for man, 0.47 g/m^2 for women).

RWT is calculated as: $\frac{IVS+PWT}{IVS+PWT+EDD}$; 0.45 is the cutoff point.

IVS = interventricular septum thickness

PWT = posterior wall thickness

EDD = end diastolic diameter of the left ventricle

LVEF will be calculated using diastolic and systolic LV volumes measured by the single-plane Simpson's rule method: ((Dvol - Svol)/Dvol) x 100; 0.50 is the cutoff point.

LV geometry is divided into four categories, based on LVMI and RWT:

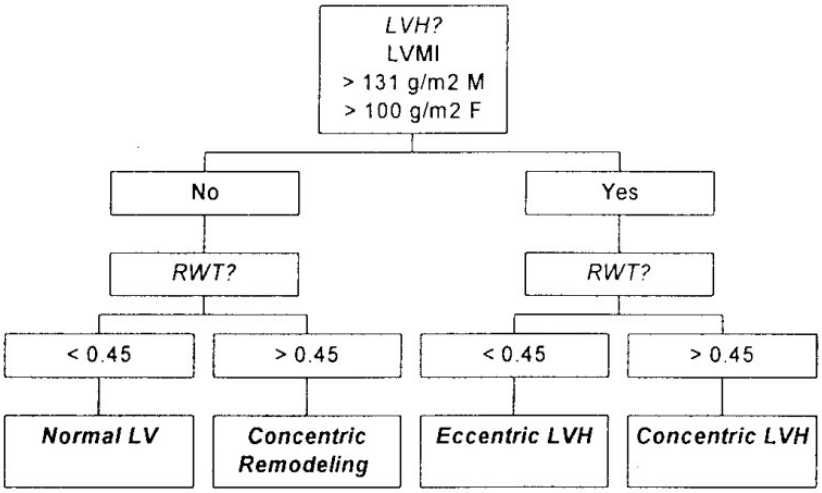

Figure 5 Left ventricle classification system of Koren et al., adapted from Middleton et al.[17]

Diastolic dysfunction is defined as an abnormality of diastolic distensibility, filling, or relaxation of the left ventricle, regardless of whether the ejection fraction is normal or abnormal and whether the patient is symptomatic or asymptomatic.[46]

4. Withdrawal of individual subjects

Participants can exit the study at any time for any reason if they wish to do so. The investigator can decide to withdraw a subject from the study for urgent medical reasons. In case of withdrawal, an end of study visit will be scheduled to perform last measurements according to visit 5. As there is no medication intervention in this study, there is no need for a safety procedure during withdrawal. Withdrawals will not be further followed.

5. Replacement of subjects withdrawn from treatment

Patients who withdraw from the study will not be replaced, as this has already been taken into account in the sample size calculation.

6. Premature termination of the study

As the interventions in this study are non-invasive and no medication is used, no specific termination criteria are formulated.

SAFETY REPORTING

1. **Temporary halt for reasons of subject safety**

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited Ethical Committee without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited Ethical Committee. The investigator will take care that all subjects are kept informed.

2. **Adverse and serious adverse events, SUSARs**

2.1 Adverse event (AE)

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational treatment. All adverse events reported spontaneously by the subject observed by the investigator or her staff will be recorded.

2.2 Serious adverse event (SAE)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death; *or*
- requires hospitalization or prolongation of existing inpatients' hospitalization; *or*
- results in persistent or significant disability or incapacity; *or*
- is a new event of the trial likely to affect the safety of the subject, such as an unexpected outcome of an adverse reaction.

All SAE's will be reported to the accredited Ethical Committee that approved the protocol.

2.3 Suspected unexpected serious adverse reactions (SUSAR)

Not applicable.

3. Follow-up of adverse events

AE according to the study are considered very unlikely, due to the fact that only non-invasive methods are used. All AEs will be followed until a stable situation has been reached and treated to standard procedures.

4. Data Safety Monitoring Board (DSMB)

Not applicable.

STATISTICAL ANALYSIS

A table with baseline characteristics of all subjects will be provided. Baseline characteristics of the population will be expressed as the mean ± standard deviation if normally distributed, or as the median [25^{th} – 75^{th} percentile] as not normally distributed, for continuous and dichotomous data, and as the frequency (%) for categorical data. Measurements of drop-outs will be included until the moment of withdrawal from the study. Normality in distribution will be tested by the Shapiro-Wilk statistic. Repeated measurements ANOVA techniques will be used for analysis. Pearson's Chi-squared test will be used to assess whether there is any distinction between subgroups in rate of kidney function decline. In case the analysis of the baseline characteristics showed any clinically relevant imbalance, we will also perform a multivariable logistic regression analysis in order to correct for this imbalance. Differences in LVH will be corrected for systolic BP and haemoglobin, as these are predictors of LVH in predialysis population.[15] Depending on the level of measurement a Chi-squared test or a T-test will be used to assess differences in secondary study parameters, and logistic and linear regression for baseline-corrected estimates. Tests will be considered significant if p-values are ≤ 0.05.

ETHICAL CONSIDERATIONS

1. **Regulation statement**

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brasil, 2013).

2. **Recruitment and consent**

All eligible patients and/or their legal representatives will receive an information leaflet by the researcher. The leaflet informs about the aims and conduct of the study, the burden and risks associated with participation. If the patient is interested in participation, he or she will mention his or her interest to the researcher, who will explain the procedure verbally in detail. Each participant will have at least one week time to decide whether he or she wishes to participate in the study. The consent and insurance procedures will be explained by the researcher. After signing the informed consent form, the study will be initiated.

3. **Objection by minors or incapacitated subjects**

Not applicable.

4. **Benefits and risks assessment, group relatedness**

Only non-invasive techniques which pose a minimal burden to the patient will be used. Blood sampling will coincide as much as possible with regular blood takings for clinical purposes. The risk of venipuncture-related complications is considered low. The burden associated with BIS and BP measurements is considered low. All subjects undergo echocardiography. Costs of this examination

will not be charged to the patient. Analysis of the echocardiography will be done during follow-up period. Aberrant results will be discussed with an expert.

5. Compensation for injury

In case of injury due participation to HORUZ study, the sponsor has a liability insurance which is in accordance with the legal requirements in Belgium: 'de wet inzake experimenten op de menselijke person van 7 mei 2004'. This insurance provides cover for damage to research subjects through injury or death caused by the study.

6. Incentives

No direct incentives will be given to the participants. None of the research examinations will be charged to the patients. For each study visit, parking ticket will be provided.

ADMINISTRATIVE ASPECTS AND PUBLICATION

1. **Handling and storage of data and documents**

All documents will be handled confidentially according to the General Data Protection Regulation. Storage of all data occurs on the 'T-schijf' of Ziekenhuis Oost-Limburg Genk.

2. **Amendments**

All amendments will be notified to the Ethical Committee that gave a favorable opinion.

3. **Annual progress report**

The investigator will submit a summary of the progress of the trial to the accredited Ethical Committee once a year. Information will be provided of the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events, other problems, and amendments.

4. **End of study report**

The investigator will notify the accredited Ethical Committee of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's visit.

In case the study is ended prematurely, the investigator will notify the accredited Ethical Committee, including the reason for the premature termination.

Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethical Committee.

5. Public disclosure and publication policy

All data that result from the HORUZ study will be made known, and will be offered for publication in peer-reviewed journals.

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Suppl.* 2013;3:1-150.

2. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney international.* Dec 2011;80(12):1258-1270.

3. Hill NR, Fatoba ST, Oke JL, et al. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. *PloS one.* 2016;11(7):e0158765.

4. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *The New England journal of medicine.* Sep 23 2004;351(13):1296-1305.

5. Matsushita K, van der Velde M, Astor BC, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet (London, England).* Jun 12 2010;375(9731):2073-2081.

6. Hung SC, Kuo KL, Peng CH, et al. Volume overload correlates with cardiovascular risk factors in patients with chronic kidney disease. *Kidney international.* Mar 2014;85(3):703-709.

7. Tonelli M, Wiebe N, Culleton B, et al. Chronic kidney disease and mortality risk: a systematic review. *Journal of the American Society of Nephrology : JASN.* Jul 2006;17(7):2034-2047.

8. Hung SC, Lai YS, Kuo KL, Tarng DC. Volume overload and adverse outcomes in chronic kidney disease: clinical observational and animal studies. *Journal of the American Heart Association.* May 5 2015;4(5).

9. Tsai YC, Tsai JC, Chen SC, et al. Association of fluid overload with kidney disease progression in advanced CKD: a prospective cohort study. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Jan 2014;63(1):68-75.

10. Tsai YC, Chiu YW, Tsai JC, et al. Association of fluid overload with cardiovascular morbidity and all-cause mortality in stages 4 and 5 CKD. *Clinical journal of the American Society of Nephrology : CJASN.* Jan 7 2015;10(1):39-46.

11. Taddei S, Nami R, Bruno RM, Quatrini I, Nuti R. Hypertension, left ventricular hypertrophy and chronic kidney disease. *Heart failure reviews.* Nov 2011;16(6):615-620.

12. Bansal N, Keane M, Delafontaine P, et al. A longitudinal study of left ventricular function and structure from CKD to ESRD: the CRIC study. *Clinical journal of the American Society of Nephrology : CJASN.* Mar 2013;8(3):355-362.

13. Foley RN, Parfrey PS, Harnett JD, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. *Kidney international.* Jan 1995;47(1):186-192.

14. Levin A. Clinical epidemiology of cardiovascular disease in chronic kidney disease prior to dialysis. *Seminars in dialysis.* Mar-Apr 2003;16(2):101-105.

15. Levin A, Singer J, Thompson CR, Ross H, Lewis M. Prevalent left ventricular hypertrophy in the predialysis population: identifying opportunities for intervention. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Mar 1996;27(3):347-354.

16. Foley RN, Curtis BM, Randell EW, Parfrey PS. Left ventricular hypertrophy in new hemodialysis patients without symptomatic cardiac disease. *Clinical journal of the American Society of Nephrology : CJASN.* May 2010;5(5):805-813.

17. Middleton RJ, Parfrey PS, Foley RN. Left ventricular hypertrophy in the renal patient. *Journal of the American Society of Nephrology : JASN.* May 2001;12(5):1079-1084.

18. Zoccali C, Benedetto FA, Mallamaci F, et al. Left ventricular mass monitoring in the follow-up of dialysis patients: prognostic value of left ventricular hypertrophy progression. *Kidney international.* Apr 2004;65(4):1492-1498.

19. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE. Impact of hypertension on cardiomyopathy, morbidity and mortality in end-stage renal disease. *Kidney international.* May 1996;49(5):1379-1385.

20. Shlipak MG, Fried LF, Cushman M, et al. Cardiovascular mortality risk in chronic kidney disease: comparison of traditional and novel risk factors. *Jama.* Apr 13 2005;293(14):1737-1745.

21. Pecoits-Filho R, Goncalves S, Barberato SH, et al. Impact of residual renal function on volume status in chronic renal failure. *Blood purification.* 2004;22(3):285-292.

22. Shafi T, Jaar BG, Plantinga LC, et al. Association of residual urine output with mortality, quality of life, and inflammation in incident hemodialysis patients: the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Aug 2010;56(2):348-358.

23. Stenvinkel P, Heimburger O, Paultre F, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney international.* May 1999;55(5):1899-1911.

24. Vilar E, Farrington K. Emerging importance of residual renal function in end-stage renal failure. *Seminars in dialysis.* Sep-Oct 2011;24(5):487-494.

25. Bellizzi V, Scalfi L, Terracciano V, et al. Early changes in bioelectrical estimates of body composition in chronic kidney disease. *Journal of the American Society of Nephrology : JASN.* May 2006;17(5):1481-1487.

26. Khan YH, Sarriff A, Adnan AS, Khan AH, Mallhi TH. Diuretics prescribing in chronic kidney disease patients: physician assessment versus bioimpedence spectroscopy. *Clinical and experimental nephrology.* Jun 2017;21(3):488-496.

27. Moissl UM, Wabel P, Chamney PW, et al. Body fluid volume determination via body composition spectroscopy in health and disease. *Physiological measurement.* Sep 2006;27(9):921-933.

28. Piccoli A. Estimation of fluid volumes in hemodialysis patients: comparing bioimpedance with isotopic and dilution methods. *Kidney international.* Apr 2014;85(4):738-741.

29. Ho LT, Kushner RF, Schoeller DA, Gudivaka R, Spiegel DM. Bioimpedance analysis of total body water in hemodialysis patients. *Kidney international.* Nov 1994;46(5):1438-1442.

30. Chertow GM, Lowrie EG, Wilmore DW, et al. Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. *Journal of the American Society of Nephrology : JASN.* Jul 1995;6(1):75-81.

31. Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *The American journal of clinical nutrition.* Apr 1985;41(4):810-817.

32. Kotler DP, Burastero S, Wang J, Pierson RN, Jr. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *The American journal of clinical nutrition.* Sep 1996;64(3 Suppl):489s-497s.

33. Covic A, Voroneanu L, Goldsmith D. Routine bioimpedance-derived volume assessment for all hypertensives: a new paradigm. *American journal of nephrology.* 2014;40(5):434-440.

34. Matsushita K, Mahmoodi BK, Woodward M, et al. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *Jama.* May 9 2012;307(18):1941-1951.

35. Sandilands EA, Dhaun N, Dear JW, Webb DJ. Measurement of renal function in patients with chronic kidney disease. *British journal of clinical pharmacology.* Oct 2013;76(4):504-515.

36. Lv J, Neal B, Ehteshami P, et al. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: a systematic review and meta-analysis. *PLoS medicine.* 2012;9(8):e1001293.

37. Agarwal R, Andersen MJ. Prognostic importance of clinic and home blood pressure recordings in patients with chronic kidney disease. *Kidney international.* Jan 2006;69(2):406-411.

38. Niiranen T, Jula A, Kantola I, et al. Home-measured blood pressure is more strongly associated with atherosclerosis than clinic blood pressure: the Finn-HOME Study. *Journal of hypertension.* Jun 2007;25(6):1225-1231.

39. Niiranen TJ, Hanninen MR, Johansson J, Reunanen A, Jula AM. Home-measured blood pressure is a stronger predictor of cardiovascular risk than office blood pressure: the Finn-Home study. *Hypertension (Dallas, Tex. : 1979).* Jun 2010;55(6):1346-1351.

40. Niiranen TJ, Jula AM, Kantola IM, Karanko H, Reunanen A. Home-measured blood pressure is more strongly associated with electrocardiographic left ventricular hypertrophy than is clinic blood pressure: the Finn-HOME study. *Journal of human hypertension.* Oct 2007;21(10):788-794.

41. Bansal N, McCulloch CE, Rahman M, et al. Blood pressure and risk of all-cause mortality in advanced chronic kidney disease and hemodialysis: the chronic renal insufficiency cohort study. *Hypertension (Dallas, Tex. : 1979).* Jan 2015;65(1):93-100.

42. Levin A, Djurdjev O, Beaulieu M, Er L. Variability and risk factors for kidney disease progression and death following attainment of stage 4 CKD in a referred cohort. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Oct 2008;52(4):661-671.

43. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian journal of psychological medicine.* Apr 2013;35(2):121-126.

44. Chamney PW, Wabel P, Moissl UM, et al. A whole-body model to distinguish excess fluid from the hydration of major body tissues. *The American journal of clinical nutrition.* Jan 2007;85(1):80-89.

45. Machek P, Jirka T, Moissl U, Chamney P, Wabel P. Guided optimization of fluid status in haemodialysis patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* Feb 2010;25(2):538-544.

46. Aurigemma GP, Gaasch WH. Clinical practice. Diastolic heart failure. *The New England journal of medicine.* Sep 9 2004;351(11):1097-1105.

APPENDIX: HORUZ REGISTRATION FORM

Studynumber: HZOL …

Age …. y
Gender M / F
Height …. cm
Smoking status no / yes / smoking cessation date: …
Etiology CKD ….
Hypertension no / yes
Diabetes no / yes
CV disease no / yes: ….
Tx list no / yes

Visit *number* *date*	*complains**	*weight*	*office BP§*	*oedeem***	**BIS thoracaal ….** **BIS whole body** *TBW / ECW / ICW* *OH / LTM / FTM*	**Cardiac function** *ECG* *Echo cor*	**Medication**	**SF36**	**homeBP¥** *morning* *evening*	**Kidney function** *eGFR$_{creat}$* *CrUrCl* *24h urine*	**CV event** **Hospitalisation** **AE/ SAE**
0 ……..	…	…	…	…	……. / …….. / …….. ……. / …….. / ……	…	…	…	… …	…	
1						Date echo cor:				… …	… … …
2 ……..	…	…	…	…	……. / …….. / …….. ……. / …….. / ……	…	…		… …	… … …	… … …
3 ……..	…	…	…	…	……. / …….. / …….. ……. / …….. / ……	…	…		… …	… … …	… … …

4 ……..	…	…	…	…	……. / …….. / …….. ……. / …….. / ……..	…	…		… …	… … …	… … …
5/End ……..	…	…	…	…	……. / …….. / …….. ……. / …….. / ……..	… Date echo cor:	…	…	… …	… … …	… … …

Abbreviations: AE adverse event, BIS bioimpedance spectroscopy, BP blood pressure, ECW extracellular water, ICW intracellular water, FTM fat tissue mass, LTM lean tissue mass, OH overhydratie, TBW total body water, SAE severe adverse event

§ office BP: BP measured during visit

¥ home BP: mean of home measurements

*complains: dyspnoe 1, orthopnoe 2, druk op de borst 3, palpitaties 4, pijnlijke gezwollen voeten 5, spierkrampen 6, duizelig 7, dorst 8, obstipatie 9, anders 10

**perifeer oedeem: geen 1, matig 2, veel 3

AE: any undesirable experience occurring to a subject during the clinical trial, whether or not considered related to the investigational treatment.

SAE: results in death; *or* requires hospitalization or prolongation of existing inpatients' hospitalization; *or* results in persistent or significant disability or incapacity; *or* is a new event of the trial

likely to affect the safety of the subject, such as an unexpected outcome of an adverse reaction