Cardiovascular rEmodelling in living kidNey donorS with reduced glomerular filtration rate: rationale and design of the CENS study

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ABSTRACT

Purpose: Until recently, it has been believed that donating a kidney not represents any risk for development of cardiovascular disease. However, a recent Norwegian epidemiological study sug- gests that kidney donors have an increased long-term risk of cardiovascular mortality. The pathophysiological mechanisms linking reduced kidney function to cardiovascular disease are not known. Living kidney donors are screened for cardiovascular morbidity before unilateral nephrectomy, and are left with mildly reduced glomerular filtration rate (GFR) after donation. Therefore, they represent an unique model for investigating the pathogenesis linking reduced GFR to cardiovascular disease and cardiovascular remodelling. We present the study design of Cardiovascular rEmodelling in living kidNey donorS with reduced glomerular filtration rate (CENS), which is an investigator-initiated prospective observational study on living kidney donors. The hypothesis is that living kidney donors develop cardiovascular remodelling due to a reduction of GFR.

Materials and methods: 60 living kidney donors and 60 age and sex matched healthy controls will be recruited. The controls will be evaluated to fulfil the Norwegian transplantation protocol for living kidney donors. Investigations will be performed at baseline and after 1, 3, 6 and 10 years in both groups. The investigations include cardiac magnetic resonance imaging, echocardi- ography, bone density scan, flow mediated dilatation, laser Doppler flowmetry, nailfold capillaro- scopy, office blood pressure, 24-h ambulatory blood pressure, heart rate variability and investigation of microbiota and biomarkers for inflammation, cardiovascular risk and the cal- cium-phosphate metabolism.

Conclusions: The present study seeks to provide new insight in the pathophysiological mecha- nisms linking reduced kidney function to cardiovascular disease. In addition, we aim to enlighten predictors of adverse cardiovascular outcome in living kidney donors. The study is reg- istered at Clinical-Trials.gov (identifier: NCT03729557).

KEYWORDS
Living kidney donors; cardiovascular remodelling; cardiovascular risk; glomerular filtration rate; chronic kidney disease; vascular calcification; microcirculation

Introduction and rationale

Chronic kidney disease (CKD) is defined as persistent (>3 months) reduced kidney function (Glomerular fil- tration rate (GFR) < 60 mL/min/1.73m^2) and/or per- sistent markers of kidney damage, such as albuminuria [1]. CKD is highly prevalent and strongly associated with an increased risk of cardiovascular disease and mortality [2]. Increased cardiovascular and all-cause mortality is seen from an early stage of CKD, when GFR declines below approximately 75 mL/min/1.73 m^2, and a multiplicative risk is seen with albuminuria [2]. Overall, patients with early stages of CKD are much more likely to die than to progress to kidney failure [3]. In patients on dialysis,

the risk of cardiovascular death is ten-20 times higher that of the general population [4]. More than 40% of the mortality in end-stage renal disease is attributable to cardiovascular disease, mostly due to non-athero- sclerotic heart disease such as sudden cardiac death, arrhythmias and congestive heart failure [5]. In patients with advanced CKD, cardiovascular remodel- ling is frequent and includes calcification of vessels [6] in addition to myocardial hypertrophy and fibrosis [7]. It has been found that more than 70% of patients starting dialysis have signs of left ventricular hyper- trophy (LVH) [8]. Recent studies indicate that the remodelling process starts at early stages of CKD [9]. Edward et al. reported reduced systolic function and increased myocardial fibrosis in CKD patients with a mean GFR value of about 50 mL/min/1.73m^2 using echocardiography and T1 mapping magnetic resonance imaging (MRI), respectively [9]. This indicates that early stage CKD may contribute to the development of cardiovascular disease. The causal relationship between reduced GFR and the development of cardiovascular disease is, however, unclear as patients with CKD often have comorbidities that contribute to cardiovascular remodelling [10]. Nevertheless, in meta-analyses con-trolling for other cardiovascular risk factors, such as diabetes mellitus and hypertension, CKD remains inde- pendently associated with cardiovascular risk [11,12].

Kidney transplantation is considered the best treat-ment option for patients with end-stage renal disease [13], preferably with a living donor graft as this offers superior graft quality and graft survival compared to kidneys from diseased donors [5]. After unilateral nephrectomy, living kidney donors experience an acute 50% reduction in GFR. After a few months, GFR grad- ually rises to reach 70% of pre-donation value due to hypertrophy of the remaining kidney [14]. Meticulous health screening programmes ensure that living kidney donors are healthy before donation, to mitigate pos- sible risks incurred by unilateral nephrectomy. Thus, living kidney donors represent a population with lim- ited confounding comorbidity, ideal to study the effects of early stage reduced kidney function on cardiovascu- lar remodelling and cardiovascular outcomes. Such studies are pertinent not only to clarify potential long- term risks for living kidney donors, but also to inform on prognosis for patients with CKD in general, and potentially identifying novel targets for CKD-directed cardiovascular therapy.

Previous epidemiological studies have not found increased cardiovascular or all-cause mortality in the follow-up of living kidney donors [15–20], and thus, kidney donation has been considered safe. However, most studies are retrospective and have compared kidney donors with a control group from the general population [15,16,20], which may bias the results as the living kidney donors are selected to be above average healthy. A few studies have, however, used control groups fulfilling the criteria for being kidney donors [17–19,21]. Most of these studies did not find increased risk for cardiovascular disease in kidney donors [17–19], but the follow-up time was short, approximately 6 years. In 2014 Mjøen et al. could demonstrate increased risk of all-cause and cardiovas- cular mortality in kidney donors after a median fol- low up time of 15 years compared to a control group also fulfilling the criteria for being a donor [21]. Moreover, this increased mortality did not emerge until 10 years post donation. These findings again indicate that there may be a connection between reduced GFR and cardiovascular disease, but the potential mechanisms are not known. Several patho- physiological mechanisms have been proposed, and we will discuss some of these which we aim to inves- tigate in the present study.

Hypertension, which in the general population is associated with an increased risk of cardiovascular mortality [22], is one of the proposed mechanisms of increased cardiovascular risk in patients with CKD. Studies regarding development of hypertension in patients due to reduced GFR after unilateral nephrec-tomy have, however, reported inconsistent results. In a meta-analysis from 2006 including 48 studies with 5145 donors a possible systolic blood pressure increase of 6 mmHg more than anticipated in the gen-eral population within 5–10 years after donation was found [23]. However, most of the studies were retro-spective and about 30% of donors were lost to follow- up [23]. In addition, most of the controls were not included simultaneously with donors [23]. Also, there is a risk of surveillance bias since the donors were fol- lowed more closely than the controls. A prospective study by Kasiske et al. in 2015 showed no significant difference in visit and 24-h ambulatory blood pressure 3 years after donation, comparing 126 kidney donors with a control group of 135 individuals [24]. In con- trast, an increased risk of being diagnosed with hyper- tension in a donor group of 1278 individuals after a median follow-up of 6.2 years compared to healthy controls, has been reported [19]. Thus, hypertension might be a contributing factor to development of car-diovascular disease in patients with CKD.

LVH is a cardiovascular risk factor of great signifi-cance [25]. Studies have shown association between reduced kidney function and LVH; both independent
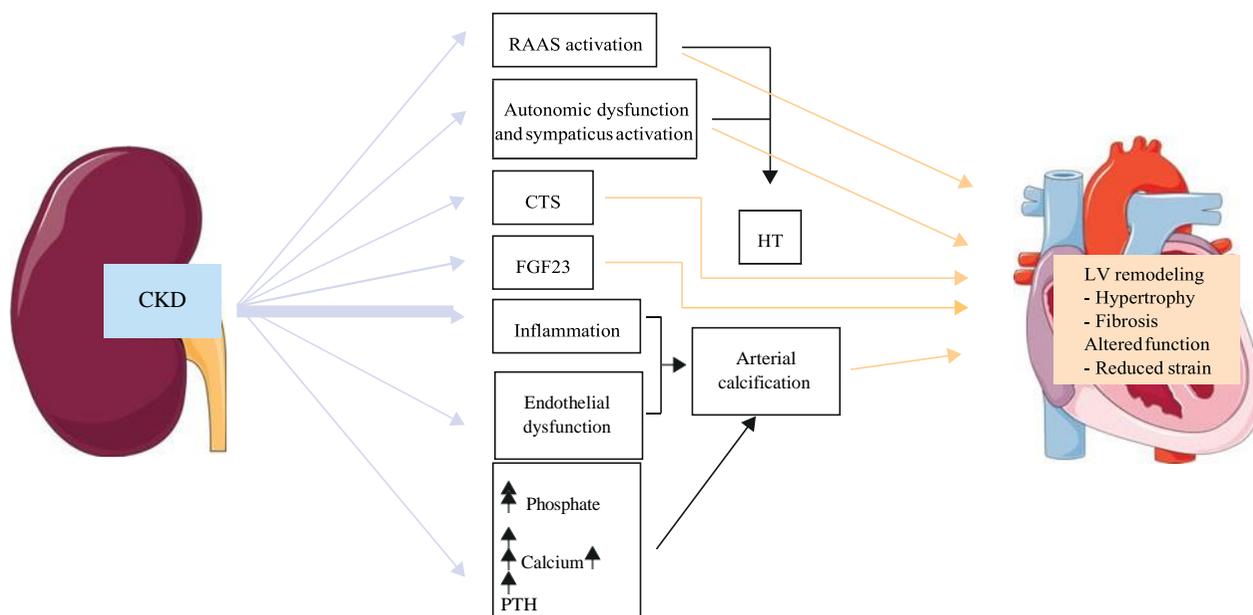
Figure 1. Potential pathophysiological mechanisms of cardiovascular remodelling in CKD patients. Abbreviations: CKD: chronic kid- ney disease; RAAS: renin-angiotensin-aldosterone-system; CTS: cardiotonic steroids; FGF23: fibroblast growth factor 23; PTH: para- thyroid hormone; HT: hypertension; LV: left ventricle. The figure contains elements modified from Servier medical by Servier, which is licensed under a Creative Commons Attribution 3.0. Unported License.

of [26], and associated, with hypertension [27]. Also, a study of 31 kidney recipients found reversal of LVH after improvement of renal function post transplant- ation [28]. A few studies have reported altered cardiac structure and/or function after unilateral nephrectomy [29–31]. Both Altmann et al. and Moody et al. found increased left ventricular mass corresponding to the reduction in GFR without an increase in blood pressure 1 year after unilateral nephrectomy [29,30], suggesting that the remodelling process takes place through other pathophysiological mechanisms than hypertension.

In addition to cardiac remodelling, CKD is associated
with accelerated medial and intimal calcification [6], representing accelerated arteriosclerosis and atheroscler- osis, respectively. Hyperphosphatemia, hypercalcemia and increased levels of parathyroid hormone might con- tribute to medial calcification by causing a shift from vascular smooth muscle cells to osteoblast-like cells [32]. Vascular calcification leads to arterial stiffening, which again contributes to development of fibrosis and LVH. Inflammation and endothelial dysfunction are also sug- gested to be important factors contributing to develop- ment of vascular calcification. Both inflammation [33] and endothelial dysfunction [34] are associated with end-stage renal disease, and proposed to contribute to increased cardiovascular mortality [35,36].
Neurohormonal mechanisms such as inappropriate activation of the renin-angiotensin-aldosterone- system are proposed as potential contributing

Factors in the development of cardiovascular disease in CKD.

Medication that inhibits the renin-angiotensin- aldoster- one-system have shown to reduce development of left ventricular remodelling in patients with essential hyper- tension [37] and the risk of cardiovascular events in patients with renal insufficiency [38]. In patients with CKD, angiotensin converting enzyme inhibitors reduce the risk for end- stage renal disease, cardiovascular events and all- cause mortality [39]. Circulating levels of renin and aldosterone have not been found elevated after kid- ney donation [29,31]. However, in addition to the circula- tory effects of the renin-angiotensin- aldosterone-system activations, studies have also suggested the importance of intracellular renin- angiotensin-aldosterone-system effects [40]. Urinary angiotensinogen has been proposed as a novel biomarker of the intrarenal renin-angiotensin- aldosterone-system and a study of 20 living kidney donors found increased levels of urinary angiotensinogen 12 months after unilateral nephrectomy [41]. Also, the autonomic nervous system and the kidneys are known to interplay [42]. Autonomic dysfunction has shown to associate with cardiovascular remodelling [43] and increased risk of early death in patients with end-stage renal disease [42].

Fibroblast growth factor 23 (FGF23) is another potential factor that might contribute to development of LVH in CKD [44]. FGF23 is synthesised in response to increased levels of phosphate and binds to the FGF receptor and its associated co-receptor

Inclusion criteria:
- Age > 18 years
- Donor group: accepted as living kidney donor at Oslo University Hospital, Rikshospitalet
- Control group:
  - Individuals evaluated for donation, but not found eligible due to non-medical causes
  - Family members related to donors or recipients

Exclusion criteria:
- Previous CVD or malignant disease (except carcinoma in situ)
- BP:
  - Office BP ≥ 140/90 mmHg or 24 hour BP ≥ 130/80 mmHg
    - Age > 60: mild HT without organ manifestations is acceptet.
- BMI:
  - Age < 30 years: BMI ≥ 30 kg/m^2
  - Age > 30 years:
    - Men: ≥ 31 kg/m^2
    - Women ≥ 32 kg/m^2
- mGFR:
  - Age < 50 years: < 90 mL/min/1.73m^2
  - Age 50-60 years: < 130-age mL/min/1.73m^2
  - ≥ 70 years: < 60 mL/min/1.73m^2
- Proteinuria:
  - Urinary albumin/creatinine ratio > 300 mg/mmol/24 hours
  - Age < 60 years with urinary albumin/creatinine ratio > 30 mg/mmol/24 hours
- Blood glucose:
  - Diabetes
  - Age < 60 with reduced glucose tolerance test
- Pathological spirometry
- Pathological stress test ECG at age > 40
- Pathological chest X-ray
- HIV, TB, HBV, HCV, EBV, syphilis and toxoplasmosis

Figure 2. Inclusion and exclusion criteria. Abbreviations: CVD: cardiovascular disease; BP: blood pressure; BMI: body mass index; mGFR: measured glomerular filtration rate; ECG: electrocardiogram; HIV: human immunodeficiency virus; TB: tuberculosis; HBV: hepatitis B; HCV: hepatitis C; EBV: Epstein-Barr virus.

Klotho in renal tubular cells [44]. Activation of the FGF receptor complex causes excretion of phosphate in urine by inhibiting reabsorption of phosphate [44]. CKD is associated with elevated circulating levels of FGF23, which increase in early stages of CKD, even before phosphate and parathyroid hormone levels increase [44]. Studies have reported on an association between FGF23 and LVH in humans [44], including kidney donors [29]. In patients starting haemodialysis, FGF23 levels were found to be independently associ- ated with mortality [45]. The FGF receptor cofactor, Klotho, is produced mainly by the kidneys. Circulating klotho, which protects the heart against stress induced maladaptive hypertrophy and remodel- ling, has been shown decreased in patients with CKD [44]. Thus, both FGF23 and klotho might play a role in cardiac remodelling during CKD.

Another interesting group of signalling molecules are the cardiotonic steroids. Cardiotonic steroids plasma levels are increased in patients with CKD and elevated cardiotonic steroids plasma levels are associ- ated cardiovascular remodelling [46]. Cardiotonic ste- roids inhibit the Na^b/K^b-ATPase and thus might increase intracellular cardiomyocyte Na^b and Ca^{2b} concentrations [46]. High intracellular cardiomyocyte Ca^{2b} concentrations are associated with increased risk of arrhythmias and sudden cardiac death in patients with heart failure [47] (Figure 1).

The aim of the CENS study is to investigate the above-mentioned potential pathophysiological mech- anisms of cardiovascular remodelling potentially induced by a reduction of GFR, utilising living kid- ney donors who experience an acute drop in kidney function, compared to age matched subjects with normal kidney function. We also aim to identify pre- dictors of adverse cardiovascular remodelling in these patients. This can help us to better select appropriate kidney donors, to give the donors cor- rect information about the consequences of kidney donation, and perhaps pinpoint important risk reducing factors.

Methods and design

Study population and design

The project is a prospective longitudinal parallel group study including subjects accepted as living kid- ney donors according to the Norwegian donor criteria at Oslo University Hospital – Rikshospitalet, Norway
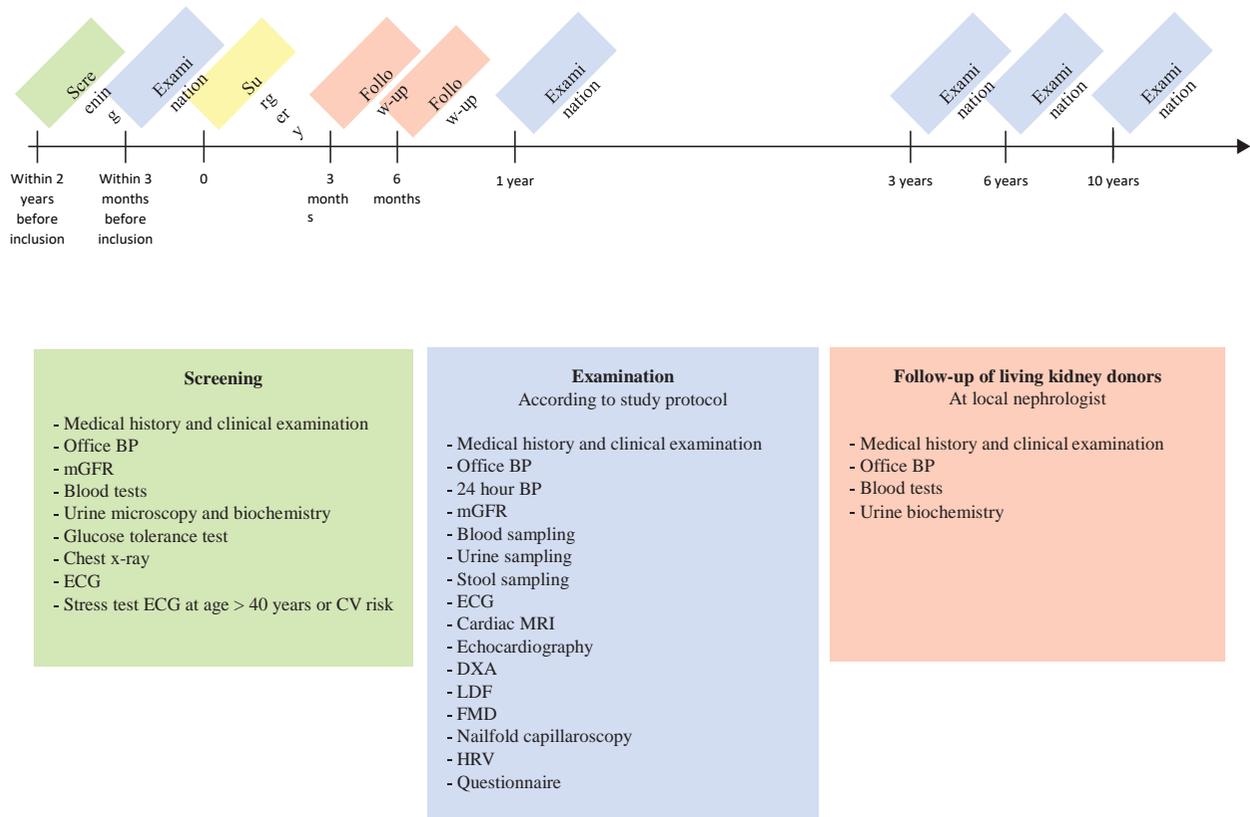
Figure 3. CENS study timeline. Abbreviations: BP: blood pressure; mGFR: measured glomerular filtration rate; ECG: electrocardio- gram; CV: cardiovascular; MRI: magnetic resonance imaging; DXA: dual-energy X-ray absorptiometry; LDF: laser Doppler flowmetry; FMD: flow mediated dilatation; HRV: heart rate variability.

(OUH), Norway's national transplantation centre. Collaboration with local nephrologist at all regional and university hospitals in Norway has been estab- lished and the project therefore has the opportunity to invite all Norwegian living kidney donors in the inclusion period to participate in the study. OUH annually performs 70–90 transplantations using living kidney donors. The 2- year inclusion period is antici- pated to result in 60 participating donors. In order to correct for naturally occurring age related changes in the cardiovascular system, the donors will be com- pared to an age- and sex-matched control group. Individuals evaluated for donation, but not found eli- gible due to causes not related to GFR and cardiovas- cular risk, such as immunological mismatch, will be invited to participate in the control group. The con- trol group will also include healthy family members related to donors, recipients and/or patients with CKD. Candidate family members will also be eval- uated according to the Norwegian transplantation protocol for living kidney donors, and must fulfil these criteria before inclusion as controls. Control individuals that accept to participate will go through the same test battery as living kidney donors. All par- ticipants will give written consent before inclusion (Figure 2).

Investigations according to the project protocol will be performed within 3 months before unilateral nephrectomy. Donors and controls will be reassessed after 1, 3, 6 and 10 years with the same test battery as at enrolment. In addition, the donors are followed up at 3 and 6 months after surgery by a nephrologist at their regional hospital, according to the transplant- ation protocol (Figure 3).

Power analysis

Our study population and study design have similar- ities to the study by Edwards et al [9], using cardiac MRI parameters in a group of CKD patients and con- trols. Based on data from the Edwards study, a power of 80% and $a < 0.05$, we need to include 50–60 indi- viduals in each group. Thus, inclusion over a period of 2 years should provide a sufficient number of par- ticipants. The inclusion of control persons might be challenging and represent a risk to the project. However, risk mitigation is achieved by combining groups mentioned above, and if necessary, use the Norwegian population registry and draw individuals for screening according to the transplantation proto- col. If the criteria are fulfilled, the individuals can be included in the control group.

Study protocol

Office blood pressure

Office blood pressure will be measured using validated automatic devices. The cuff size will be adjusted according to the arm circumference. Measurements will be performed after 5 min of rest and with the participant having refrained from tobacco, exercise and caffeine intake for the past 30 min. Measurements will be performed with the participant in a relaxed, sitting position, without crossing legs and with the cuff at heart level in a quiet room with an observer present. First, blood pressure will be measured in both arms. The arm with the highest blood pressure will be used for all subsequent measurements. Blood pressure will be measured three times with intervals of at least 1 min. Additional measurements (max-imum two) will be recorded if the last two readings differ by > 10 mmHg in either systolic, or diastolic, blood pressure. The blood pressure value is deter- mined as the average of the two last measurements.

24 Hour blood pressure

Measurements will be done on the non-dominant arm. The cuff size will be adjusted according to the arm circumference. Blood pressure will be recorded automatically every 20 min during the day (between 07 and 22) and every 60 min at night (between 22 and 07). A minimum of 70% usable blood pressure readings is required. The participants will be informed to 1) fill out a diary with information about special events that occur and sleep quality, 2) keep the arm at rest and avoid movement at every blood pressure measurement, 3) do common everyday activ- ities, but avoid heavy physical activity.

Cardiac magnetic resonance imaging

Detailed assessment of myocardial structure and func- tion will be performed with a 1.5 T scanner (Philips Ingenia; Philips Healthcare, Best, The Netherlands) utilising several methods. A stack of short axis CINE loops as well as long axis, four chamber and left ven- tricular outflow tract CINE loop will facilitate robust measurements of global function and structural parameters, such as left ventricular and right ventricu- lar mass, stroke volumes and ejection fractions. Native T1-mapping will be used to evaluate myocar- dial fibrosis patterns. Tissue phase mapping will be used to measure the detailed 3 D deformation of the heart, allowing comprehensive analysis of regional myocardial strain and strain rate with high resolution.

Thus, tissue phase mapping offers information about both systolic and diastolic function. Moreover, in combination with blood pressure, tissue phase map- ping allows measurement of regional myocardial work [48]. Also, the combination of T1-mapping and tissue phase mapping enables high sensitive assessment of myocardial structure-function relationships.

Pulse wave velocity is a well-established marker for aortic stiffness and is a strong and independent pre- dictor of cardiovascular mortality in patients with end-stage renal disease [49]. Assessment of aortic pulse wave velocity using flow-encoded MRI will be used to measure aortic stiffness [50].

Echocardiography

Standard and 3 D-echocardiography will be performed using a Vivid E9 cardiac ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway). Myocardial function and structure will be followed over time by B-mode, M-mode, tissue doppler, blood flow doppler and strain imaging, as previously described [51].

Carotid ultrasound

Non-occlusive atherosclerotic plaques and increased carotid intima media thickness represent early vascu- lar remodelling and is associated with increased car- diovascular risk [52]. Carotid intima media thickness will be measured after recommendations by Touboul et al. [53]. Carotid ultrasound in B-mode will be used, with a (6–15.0 MHz) linear array transducer (ML6-15-D) at frequencies >7 MHz. The depth of focus will be about 30–40 mm and the optimal frame rate about 25 Hz (>15 Hz). The common carotid artery, the carotid bulb and the internal carotid artery will be visualised on both sides in longitudinal and cross-sectional views. The carotid intima media thick- ness measurement will be performed with a lateral probe position on both sides in a longitudinal view, perpendicular to the ultrasound beam with both walls clearly visualised in a region free of plaques on the far wall of the common carotid artery, at least 5 mm below its end. An automated system will be used to provide accurate measurements of carotid intima media thickness and common carotid artery inter-adventitial and intraluminal diameters.

Heart rate variability

The function of the autonomic nervous system regu- lating the sinoatrial node can be assessed with heart rate variability [54]. Low heart rate variability is asso- ciated with increased mortality in patients after acute

myocardial infarction [55]. Heart rate variability will be measured using a Polar V800 monitor [56]. RR interval data will be recorded continuously during night-time at a sampling frequency of 1000 Hz.

Nailfold capillaroscopy

Microvascular architecture can be assessed easily and non-invasively using nailfold capillaroscopy. Changes in nailfold capillary architecture might represent changes in the microvascular architecture in other organs, including the heart. Therefore, we will per- form nailfold capillaroscopy to evaluate changes in the microvascular architecture due to a reduced GFR. Nailfold capillaroscopy will be performed after recom- mendations by Etehad Tavakol et al. [57]. The partici- pants will be asked to avoid smoking, caffeine intake and vigorous physical activity the last 6 h before examination. They will be seated in a quiet room with temperature of 20–24 °C. Capillary density meas- urements will be performed with nailfold microscopy using a 200 × magnification capillaroscope (Dino-Lite Pro 200×). Finger number 3, 4 and 5 on both hands will be examined and three pictures will be taken of each finger. In all of the 18 pictures, the number of capillaries per millimetre will be measured, using the DinoCapture 2.0 software. The capillary density is determined as the mean of the 18 measured densities.

Laser Doppler flux and flow mediated dilatation

Laser Doppler flux enables non-invasive monitoring of the microvascular blood perfusion of the skin. The vascular endothelium possesses a potent vasodilatory mechanism lowering vascular resistance, which is believed to represent a general function of the micro- circulation, including vessels of the heart [58]. Flow mediated dilatation enables non-invasive investigation of endothelial function [59]. We will utilise laser Doppler flux and flow mediated dilatation simultan- eously to investigate endothelial function in small and medium-sized vessels, respectively. Flow mediated dilatation will be performed according to current guidelines [60].

The participants will be investigated in a quiet, darkened, room, resting in a supine position. The par- ticipants must have avoided vigorous physical activity, and be fasting (including smoking, alcohol and caf- feine intake) since the previous evening.

The laser Doppler probes (moorVMS, Moor instru- ments, Devon, UK) will be fastened with adhesive tape to the pulps of the third fingers of both hands (dominated by the shunt vessels; arteriovenous anastomoses), and 2 cm proximal for the wrist of the dorsal site of both hands (dominated by nutritive ves- sels). An automated forearm cuff will be placed below the elbow of the right arm (moorVMS-PRES, Moor instruments, Devon, UK). After fastening of the laser Doppler probes and application of the forearm cuff, the right brachial artery will be visualised in longitu- dinal section about 5 cm proximal to the antecubital fossa with a 12 MHz linear array ultrasound probe (Zonare z.one; ZONARE Medical Systems, Mountain View, CA, USA). Simultaneously, an electrocardio- gram (ECG) will be recording and instantaneous arterial blood pressure will be obtained from the left fourth finger (Ohmeda 2300 Finapres, Madison, WI). Skin temperature and room temperature will also be recorded.

After ten min of control measurements, the fore- arm cuff will be inflated to 200 mmHg (above systolic blood pressure) for five min. After this, shear stress will be made by deflating the cuff and measurements is then continued for ten min after cuff deflation.

Recording will be done simultaneously from all four Laser Doppler probes with sampling frequency of 40 Hz. The noise limitation filter of the Laser Doppler instruments will be set to the highest level (21 kHz), and the radiated wavelength will be 820 nm. Flux output signals will be filtered at time constant 0.1 s. Baseline diameter of the brachial artery will be stored before occlusion. After deflation, brachial artery diameter will be stored for 180 s.

Dual-energy X-Ray absorptiometry (DXA)

We will evaluate changes in bone mineral density and soft tissue composition using a Lunar Prodigy (Madison, WI, USA) DXA scanner to perform DXA scans of the total body, lumbar spine and hips [61].

Specific bloodsamples

Renal function will be assessed using the renal clear- ance of iohexol following a 5 mL iv injection of Omnipaque^{v} (iohexol) 300 mg I/mL. Routine bio- chemical parameters will be recorded and analysed by conventional methods. In addition, blood samples will be processed after standardised procedure, and serum and plasma samples aliquoted and stored at —80 °C in a biobank. That also includes PaxGene tubes for gene expression analyses. A number of biomarkers, including markers of inflammation, connective tissue remodelling, bone metabolism and calcification, are planned analysed.

Faecal samples for biobank

Faecal samples will be collected using a sampling device and dry sterile tubes with spoons for fresh faeces. The samples will be aliquoted and stored dry at —80 °C within 24 h after sampling.

Urine samples for biobank

Morning urine samples will be collected and centrifu- gated at 4 °C at 2500 × g for ten min and the super- natant will be aliquoted and kept at —80 °C in a biobank.

Ethics

The protocol has been approved by the Regional Committee for Medical Research Ethics in December 2016 (ref. number: 2016/1961) and the study has been registered at Clinical-Trials.gov (identifier: NCT03729557). The participants will be included in the study after informed and written consent.

The study has a steering committee for monitoring the study progress and quality. The Steering commit- tee consisting of Consultant Jon Arne Birkeland, Professor Ivar Sjaastad, Consultant Dag Olav Dahle, PhD-candidate Kjersti Blom and a user representative from the National Association for Kidney Disease and Transplant, Olav Bjørnsund Hollingsaeter. In add- ition, we will use standardised forms for patient reported outcome measures (PROMS) that will meas- ure the health status of the participants in the study seen from a user site.

Discussion

There is strong epidemiological evidence for an asso- ciation between reduced kidney function and cardio- vascular disease [2,5]. Studies also suggest that cardiac remodelling in CKD begins early [9]. However, it is difficult to investigate the causative relationship between CKD and cardiovascular disease because of multiple confounders in this group of patients. Kidney donors represent a unique model for investi- gating the potential pathophysiological mechanisms

because they are screened for cardiovascular morbid- ity before donation and end up with 70% of their ori- ginal GFR after unilateral nephrectomy [14]. The CENS study will examine consequences of unilateral nephrectomy and the mechanisms connecting reduced kidney function to cardiovascular disease. The main outcome of the CENS study is structural and func- tional remodelling of the heart. The secondary out- come is structural and functional remodelling of the small-, medium sized and large vessels. A sub goal of the CENS study is to investigate factors that might change due to a reduction of GFR, and potentially induce changes in the cardiovascular system.

Structural and functional remodelling of the heart

LVH is common in patients with advanced kidney disease and is associated with cardiovascular mortality [8]. The presence of cardiovascular structural and functional remodelling after kidney donation has until recently been poorly explored. This is partly due to methodological aspects, as earlier studies on cardiac remodelling in CKD have been performed using con- ventional echocardiography techniques. These meth- ods do not have the proper sensitivity to detect early structural and functional alterations in the cardiovas- cular system [62]. Recent studies investigating cardiac structure and function in living kidney donors with more sensitive MRI methods suggest development of small changes in the heart after unilateral nephrec- tomy [29–31]. Moody et al. even found global left ventricular remodelling as early as 1 year after kidney donation [29]. However, the relationship between altered structure and changes in myocardial function on the regional level in kidney donors is not known. The CENS study will provide novel information about cardiac remodelling by using even more sensitive MRI techniques such as 3 D tissue phase mapping.

Native T1 mapping provides information about the microstructure of the myocardium [63], including the degree of fibrosis [64]. Several pathological processes influence T1 values, such as diffuse myocardial fibro- sis [64], oedema [65], and infiltrative diseases [66]. We hypothesise that native T1 will be altered in kid- ney donors due to development of diffuse fibrosis sec- ondary to reduced kidney function.

Native T1 mapping is often used in combination with gadolinium-based contrast agents to provide information about the extracellular volume [67] and diffuse fibrosis [68]. However, known risk factors when using gadolinium-based contrast agents include allergic reactions and nephrogenic systemic fibrosis in

patients with advanced renal disease [69]. Also, sev- eral studies have shown signs of gadolinium depos- ition in the brain after repeated exposure to gadolinium-based contrast agents, of which the conse- quences are not known [70]. Kidney donors are healthy before kidney donation, and we do not expect changes in T1 value due to other pathology such as oedema, amyloidosis or iron overload. We can there- fore assume that, if found, an increase in native T1 values in living kidney donors compared to controls are due to development of interstitial cardiac fibrosis secondary to a reduction in GFR. Moreover, the added value of post-contrast T1 compared to purely native T1 is still a matter of debate [71]. Therefore, we have chosen not to expose the study participants to gadolinium-based contrast agents.

While T1-mapping provides information about myocardial structure, tissue phase mapping offers detailed information about left ventricular function. Tissue phase mapping provides strain and strain rate with very high temporal and spatial resolution [72]. This is essential for detecting subtle changes, i.e., it offers higher sensitivity. Moody et al. [29] used fea- ture tracking, which is an MR post-processing tool used to extract deformation parameters from conven- tional CINE MR scans. However, feature tracking is less sensitive than tissue phase mapping in detecting the complex deformation of the left ventricular [73] since tissue phase mapping provides information about the direction and velocity of each point of the left ventricular myocardium for all time points. By measuring blood pressure in connection with the MRI examination, one can also calculate myocardial work by combining this with data from the tissue phase mapping investigation [48]. To our knowledge, no other study has previously investigated living kid- ney donors and a comparable control group prospect- ively using T1-mapping and tissue phase mapping. The CENS study will therefore contribute to novel information about cardiac remodelling induced by reduced GFR.

Structural and functional remodelling of the large-, medium sized and small vessels

The CENS study will also investigate arterial remodel- ling in large-, medium sized and small vessels. Thus, if present, adverse arterial remodelling will not only be identified, but also localised to different segments of the arterial system. This is important not only for peripheral remodelling, but also because it is a deter- minant of cardiac function.

Signalling mechanisms inducing cardiovascular remodelling due to CKD

Thus, we intend to investigate arterial and cardiovas- cular remodelling in living kidney donors, and further investigate the association with altered calcium- phos- phate metabolism, endothelial function, inflammation parameters and autonomic nervous system function. The aim of the CENS study is to enlighten potential pathophysiological mechanisms and predictors of car- diovascular disease in patients with CKD. As far as we are aware of, no previous study has performed such novel and extensive investigation of kidney donors and an equally healthy control group. The CENS study will contribute with novel insights regarding cardiovascular remodelling due to reduced GFR in living kidney donors.

Importance and clinical significance

This knowledge is especially important for two groups. Firstly, it might contribute to more robust risk stratification, and possibly, identify risk-reducing interventions in living kidney donors. Living kidney donors make a significant allowance by contributing to the transplantation programme. Hence, they deserve the best possible medical care, and the most updated information available regarding possible adverse effects. Second, CKD patients are at increased risk of death, cardiovascular events and hospitalisa- tion compared to the general population [74]. Whether this observed increase in risk is explained by CKD or comorbidity secondary to CKD, e.g., hyper- tension, is not properly investigated, though recent studies have suggested that reduced kidney function is an independent risk factor for development of cardio- vascular disease [29]. This project will give more information about the mechanisms underlying increased risk of cardiovascular disease in patients with reduced GFR. Such knowledge might contribute to identify risk-reducing interventions. Taken into account the large numbers of CKD patients among the general population, our findings, if they turn into interventions, might have a huge impact on pub- lic health.

Disclosure statement

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