Assessment of kidney health: implications for living kidney donors and beyond

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DOI:
10.33612/diss.784830904

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Colofon
Author: Jessica van der Weijden
Provided by thesis specialist Ridderprint, ridderprint.nl
Printing: Ridderprint
Cover design: Jessica van der Weijden
Layout and design: Inge Hattuma | persoonlijkproefschrift.nl

Financial support for the publication of this thesis was generously provided by de Nederlandse Transplantatie Vereniging (NTV) and the Rijksuniversiteit Groningen.

NEDERLANDSE
TRANSPLANTATIE
VERENIGING

ISBN: 978-94-6483-382-9

rijksuniversiteit
groningen

Assessment of kidney health: implications for living kidney donors and beyond

Proefschrift

ter verkrijging van de graad van doctor aan de Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 11-10-2023 om 16:15 uur door

Jessica van der Weijden

geboren op 10 augustus 1995 te Amsterdam

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1

CHAPTER

Introduction

BACKGROUND AND CONTEXT OF LIVING DONOR KIDNEY TRANSPLANTATION

The history of kidney transplantation goes back to 1902, when Dr. Emerich Ullmann from Austria pioneered in this field by experimenting with kidney transplantations in dogs and sheep.[1] In the next years, several failed attempts of kidney transplantation in humans followed. In 1954 the first successful human kidney transplantation was performed by Dr. Joseph Murray and Dr. David Hume at Brigham Hospital in Boston, U.S.A. among two identical twins[2] From then, the field of kidney transplantation developed rapidly, especially with the development of immunosuppressive drugs.[3–5] Prevention of graft rejection by immunosuppressive therapy made it possible to transplant kidneys across immunological barriers, increasing possibilities for patients to find a donor. International exchange registries for deceased donation were established, and options for living kidney donation were expanded to non-relatives, either directly or through "paired exchange" programs, and anonymous living donation became possible. The global proportion of living donors was 41% in 2015, but this percentage varies substantially between different countries.[6,7] In the Netherlands, 50% of transplanted kidneys come from living donors.[8] Despite these important positive developments over the past decades, the pressure on transplant programs remains immense due to an ongoing shortage of available donor kidneys. Currently, there is no prospect of a decrease in the demand for kidney replacement therapy, because the global prevalence of chronic kidney disease (CKD) – currently 9-13% – is rising.[9,10] This increase is mainly driven by the ageing population, as well as increasing prevalences of diabetes and obesity.[9]

Besides the significant contribution to the number of available kidney allografts, living donor kidney transplantation brings more advantages. The possibility to transplant pre-emptively (i.e., before the recipient reaches kidney failure requiring dialysis) and younger donors who are often related to the recipient result in better outcomes for the recipient.[11] Also, reduced warm and cold ischemia times due to (almost) simultaneous donation and implantation procedures in the same hospital as well as planning of the procedures during daytime instead of the (nighttime) emergency setting are aspects of living donation that are believed to contribute to favorable outcomes for recipients.[11] Recently, also recipient-related characteristics such as social support (which may be better in recipients that are able to find a living donor) have been proposed that may drive the favorable effect of living donation on transplantation outcomes.[12] The major disadvantage of living kidney donation, however, is that completely healthy individuals undergo

major surgery to benefit the health of other people, who are sometimes complete strangers to them. This may be at the cost of the donor´s own health, and even though risks of kidney failure after living kidney donation do not exceed risks of the general population,[13–15] the risks in fact are detectably higher when compared to matched healthy non-donors (absolute risk around 30 per 10.000 donors, ten-fold increase compared to healthy non-donors).[16–19] This brings an ongoing ethical dilemma in which donor risks must constantly be weighed against the benefits. When denying a donor to donate, the potential harm of taking away the opportunity for the donor to improve survival chances of the recipient, often a spouse or close relative, should be taken into consideration.[20] The procedure is generally considered ethically justified because the minimal risks that living kidney donors face do not outweigh the harm that can be prevented by the procedure.[20,21] Yet, in order to enable informed decision making in living kidney donor selection, pre-donation risk assessment is of utmost importance.

Assessment of kidney function

One of the most important elements of living kidney donor evaluation is kidney function assessment. The main aim of kidney function assessment in the donor is to determine whether they will retain sufficient kidney function after donation, but also to determine whether the donor kidney will have sufficient function for the recipient.[22,23] Glomerular filtration rate (GFR) in mL/min, usually normalized for body surface area (BSA) to mL/min/1.73m^2, is considered the best index for kidney function.[24] There are several measurement and estimation methods that are considered to give a close approximation of the true GFR. Determination of GFR requires a marker that has the properties of being water-soluble, unbound to protein, freely filtered by the glomerulus as well as not being reabsorbed and/or secreted in the tubules. Ideally, no extrarenal clearance occurs. Measuring the clearance of exogenous filtration markers (e.g. inulin, iohexol or iothalamate) is the gold standard. It is done by intravenous infusion of the tracer at a constant rate, after which several blood and/or urine samples are collected over a period of a few hours to calculate how much of the tracer is eliminated from the blood by the kidneys. The GFR is then calculated as:

$$GFR = (U \times V) / P$$

Where U and P represent the urinary and plasma concentrations of the tracer and V represents the urinary flow rate in mL/min. To account for the circadian rhythm of GFR, the most ideal measurement period would be even 24 hours.[25]

Disadvantages of this "direct" method to measure GFR are that it is time-consuming and costly and therefore less suitable for routine use in clinical practice.[24]

Alternatively, the GFR can be estimated using endogenous filtration markers, of which serum creatinine and serum cystatin C are most widely used. Creatinine is a biologically inert waste product of creatine metabolism in muscles, and non-GFR determinants include age, sex and body size.[26,27] Also, both serum creatinine and creatinine excretion can be increased with high meat intake.[27] Creatinine is freely filtered by the glomerulus, and therefore its clearance can be determined by calculating how much creatinine is excreted in urine over a period of 24 hours divided by the plasma level of creatinine. Measurement of 24-hour creatinine clearance is not perfect because next to glomerular filtration, 10% of creatinine that is excreted in the urine is secreted by the proximal tubule. This results in an overestimation of GFR, especially in the lower ranges, since tubular creatinine excretion plays a more prominent role in the lower GFR range. Moreover, measurement error can easily be introduced by urine over- or undercollection.[27] A more practical method is estimation of GFR (eGFR) using an equation that includes serum creatinine and covariates to adjust for other non-GFR-driven determinants of serum creatinine concentrations. The Chronic Kidney Disease Epidemiology (CKD-EPI) equation is currently the most widely used equation and has variants based on serum creatinine, cystatin C or a combination of both.[28–30] Cystatin C is a cysteine protease inhibitor that is produced by all nucleated cells in the human body at a constant rate.[31] Like serum creatinine, cystatin C is freely filtered by the glomerulus, but 99% is reabsorbed in the proximal tubule where it is catabolized, and no tubular secretion of cystatin C occurs afterwards.[32] Cystatin C concentrations are less dependent on body composition and nutritional status, but non-GFR determinants include all that affect general cellular metabolism such as inflammation or hyperthyroidism.[33,34] Other non-GFR determinants of cystatin C that have been described include obesity and smoking.[33–36] A limitation of creatinine- and cystatin C-based eGFR equations is that they underestimate mGFR in living kidney donors because they have been developed in populations with lower GFR.[37–42]

Because each of the GFR assessment methods discussed above have their own limitations, there is no consensus on the ideal method to assess GFR in potential donors, despite its prominent role in donor screening. The recommendations on GFR assessment in donors by the Kidney Disease Improving Global Outcomes (KDIGO) organization, the British Transplant Society/UK Kidney Association (BTS/UKKA) and the Dutch "Landelijk Overleg Niertransplantatie" (LONT) are

summarized in Table 1.[22,23,43] The Dutch guideline is mainly based on the British guideline published in 2018, whereas some recommendations have been adapted to match the Dutch situation.[43]

Table 1. Assessment of GFR in potential living kidney donors according to (inter)national guidelines.

	KDIGO	BTS/UKKA	LONT
GFR method	$eGFR_{cr}$ initially, confirmation with mGFR, 24hCrCl, $eGFR_{cr\text{-}cys}$ or repeated $eGFR_{cr}$ when $eGFR_{cr}$ is inaccurate or when more accurate GFR assessment will impact treatment decisions	$eGFR_{cr}$ initially, save from further donor evaluation when $eGFR_{cr}$<45. When eGFR>45, confirmation with mGFR	$eGFR_{cr}$ initially, save from further donor evaluation when $eGFR_{cr}$<45. When eGFR>45, confirmation with mGFR. Confirmation with 24hCrCl in centers with no mGFR
Acceptance threshold	>90 → accept 60-90 → individualize decision <60 → decline	Provides age- and sex adapted thresholds	BTS/UKKA thresholds when mGFR was used. KDIGO thresholds when 24hCrCl was used
Recommendation	When GFR is 60-90: base decision on demographic and health profile and transplant program´s acceptable risk threshold	>threshold+no kidney failure risk factors: accept; When <threshold or >threshold+kidney failure risk factors: base decision on predicted lifetime incidence of kidney failure*	BTS/UKKA advice when mGFR was used. KDIGO advice when 24hCrCl was used. When doubt about 24hCrCl result: refer to center that has mGFR testing available

*Quality of evidence for this advice was graded as "very low" by the guideline authors, the advice is a "suggestion"
Abbreviations: 24hCrCl: 24-hour creatinine clearance; BTS/UKKA: British Transplantation Society and UK Kidney Association; eGFRcr: estimated glomerular filtration rate based on serum creatinine; mGFR: measured glomerular filtration rate; KDIGO: Kidney Disease Improving Global Outcomes; LONT: Landelijk Overleg Niertransplantatie.

All guidelines agree that creatinine-based eGFR is not sufficient for final decision making, but there is no agreement as to which confirmatory test should be used. Indeed, most studies show that eGFR should be used with caution in donor screening,[37–42] and that it could even result in improper acceptance or denial of donor candidates.[44] Ideally, eGFR is confirmed by mGFR in every potential donor, but this is not feasible given the practical limitations of mGFR described above. The question is therefore in which donors eGFR fulfills and which donors benefit

from confirmatory testing. In 2016, Huang *et al*. developed an online calculator that predicts pre-donation mGFR thresholds based on age, sex, race and pre-donation eGFR (CKD-EPI) using Bayesian statistics.[45] Although it was not developed in living kidney donors, its usefulness was validated in living kidney donors by Gaillard *et al*.[46] However, since the goal of donor evaluation is to determine post-donation risks, assessing the performance of eGFR to predict post-donation mGFR would be even more contributive to informed decision making.

Another discrepancy is that the KDIGO guideline provides fixed pre-donation GFR thresholds, whereas the BTS/UKKA guideline provides age- and sex adapted thresholds. Clinical implementation of fixed thresholds for kidney function has been criticized because it does not account for physiological age-related decline of kidney function.[47,48] The thresholds provided by the BTS/UKKA guideline serve the goal of predicting a post-donation GFR value that is within the normal age-adapted GFR range, assuming that post-donation GFR compensates up to 65-75% of the pre-donation value.[23] They were age-adapted to account for the remaining life expectancy and thus the expected remaining age-related physiological function loss. However, the age-adapted normal ranges were based on cross-sectional studies and the advised pre-donation GFR thresholds were not based on actual prediction models. Moreover, determination of desired post-donation GFR is complicated because normal ranges for single-kidney GFR in healthy individuals are unknown.

Glomerular filtration rate and kidney health

The GFR is considered to be reflective of the number of functioning nephrons in the kidney, because total GFR is dependent on the total number of functioning nephrons and their single-nephron GFR, of which the latter is constant in healthy individuals <70 years.[49] After living kidney donation, however, 65-75% of the GFR remains while 50% of the functioning nephrons were removed. Since nephron formation ceases at late gestation or early after birth,[50] the increase in GFR observed after living kidney donation must be achieved by an increase in the single-nephron GFR of the remaining nephrons. Single-nephron GFR is the result of hydraulic pressure in the glomerular tuft, regulated by the afferent and efferent arterioles, the transcapillary colloid osmotic pressure gradient, and the ultrafiltration coefficient, as shown in **Figure 1**. The ultrafiltration coefficient is the result of the permeability and surface area of the glomerular filtration barrier (consisting of endothelial cells, the glomerular basement membrane, and podocytes). Besides kidney donation, risk factors for CKD including hypertension, overweight and family history of kidney failure have been associated with

a higher single-nephron GFR.[49] These associations support the assumption that when nephrons are lost, be it because of kidney donation or because of disease, the remaining non-damaged nephrons increase single-nephron GFR in order to maintain GFR. This increase in single-nephron GFR when nephrons are lost is attributed to the reserve capacity of the kidney.[51,52] Because of this assumed reserve capacity, the GFR in itself cannot always be considered a perfect reflection of the actual number of functioning nephrons.

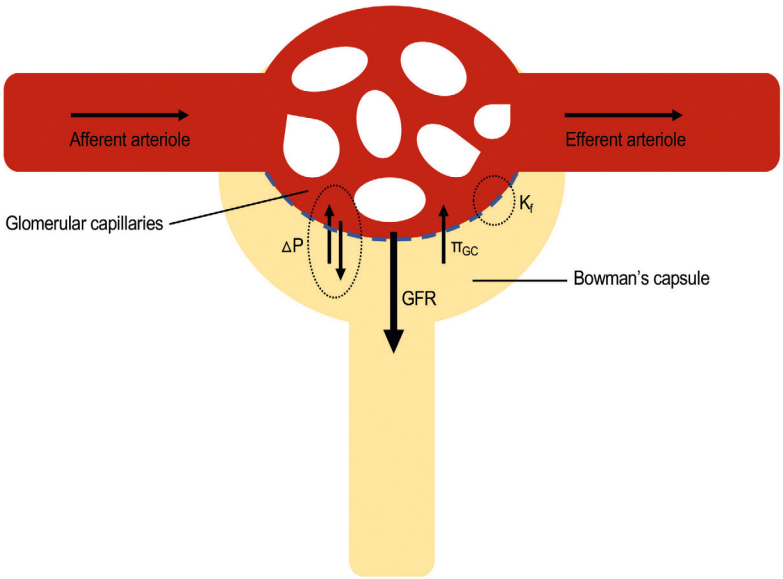

Figure 1. Schematic representation of filtration in the glomerulus.

Renal plasma flow is regulated by the afferent and efferent arterioles. The net filtration pressure gradient is determined by the hydraulic and oncotic pressures in the glomerular capillaries and the Bowman's capsule. The net hydraulic pressure gradient (ΔP) is the difference between the hydraulic pressure in the glomerular capillaries and the Bowman's capsule, in which the hydraulic pressure in the glomerular capillaries is usually greater than the hydraulic pressure in the Bowman's capsule. The oncotic pressure in the Bowman's capsule is considered to approach zero in healthy conditions, and therefore the net oncotic pressure gradient is determined by the oncotic pressure in the glomerular capillaries (π_{GC}). The GFR is determined by the net filtration pressure gradient multiplied by the ultrafiltration coefficient (K_f). The K_f is determined by the permeability and the surface of the filtration area. Thus, GFR = $(\Delta P - \pi_{GC}) \times K_f$.

Microstructural determinants of pre- and post-donation GFR

Another example that GFR might not always give an accurate estimate of kidney health is demonstrated in kidney biopsies, often taken in the post-transplant setting, where interstitial fibrosis with tubular atrophy (IF/TA) can be much more abundant than expected based on (relatively preserved) kidney function.

Alterations in kidney tissue on microstructural level might therefore provide additional information on kidney health, particularly in early stages of kidney damage when GFR is still normal. This could be valuable information for living kidney donors who may have subtle kidney damage, not captured by a reduced GFR, that might make them more prone to developing progressive loss of kidney function after donation. Studies linking pre-implantation biopsy information with donor characteristics, kidney function and post-donation outcomes showed that glomerular volume, tubular area, glomerulosclerosis and arteriosclerosis are inversely associated with nephron number and positively with single-nephron GFR.[49] Higher glomerular volume has been associated with clinical characteristics including higher blood pressure, obesity, higher total GFR, taller height and family history of kidney failure.[49,54,55,58,59] Higher age and hypertension seemed the main determinants of glomerulosclerosis.[54,55,57] A ten-year post-donation eGFR <45 mL/min/1.73m^2 was predicted by higher glomerular volume and lower nephron number.[56] Additionally, higher glomerular volume was an independent determinant of four months post-donation mGFR <60 mL/min/1.73m^2,[53] self-reported albuminuria at 10 years post-donation,[56] but not self-reported 10-year post-donation eGFR <60 mL/min/1.73m^2.[56]

These studies show that even though donors are selected based on health and normal GFR, some have microstructural alterations in nephron morphology and/or histology that correlate with risk factors for CKD and/or accelerated loss of kidney function post-donation. Especially glomerular volume seems of interest, considering its associations with nephron number, single-nephron GFR, clinical characteristics and post-donation outcomes. An increase in glomerular volume results in an increased amount of filtrate presented to the proximal tubules, which may explain the tubular hypertrophy in concordance with glomerular hypertrophy.[60] This increase in proximal tubular workload may in turn impact the peritubular capillaries. Loss of peritubular capillaries (peritubular capillary rarefaction) is a well-established phenomenon in (advanced) CKD,[61] but whether alterations in peritubular capillary density could also serve as a marker for more subtle kidney damage is unknown. The kidney is unique in comparison to other organs in the sense of organization of its microvasculature because it contains two sequential capillary beds: the glomerular and peritubular capillaries (**Figure 2**).[62,63] Capillaries react to environmental alterations such as changes in blood flow or tissue metabolic demand. Therefore, we hypothesized that peritubular capillary density could be affected by nephron hypertrophy through two pathways: through an increased tubular metabolic demand as well as through an increased peritubular capillary plasma flow as a result of increased glomerular

plasma flow. To date, the association of peritubular capillary density with glomerular and tubular morphology and histology has not been studied in healthy individuals with no clinical signs of kidney disease. Capillary rarefaction in donors with altered glomerular and tubular morphology may potentially be an adverse prognostic sign for post-donation outcomes.

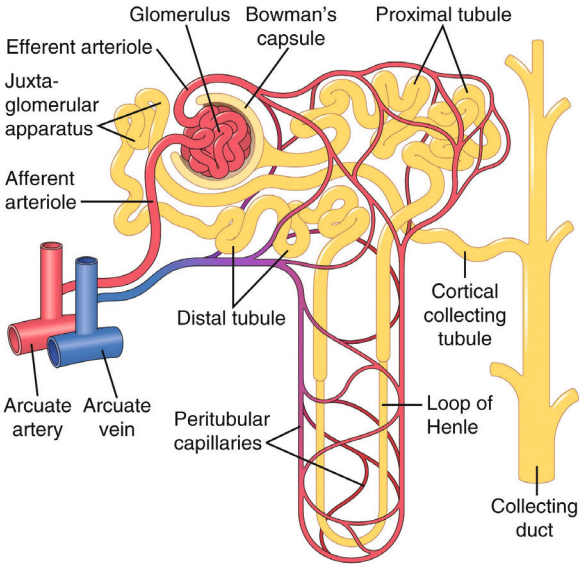

Figure 2. Schematic representation of the microcirculation of a nephron.

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Post-donation compensation of GFR

The general understanding of glomerular hypertrophy and an increased single-nephron GFR has been that over time, hyperfiltration leads to podocyte detachment, proteinuria, tubulointerstitial inflammation, nephrosclerosis and a subsequent vicious circle of more hyperfiltration and nephron loss.[60,64,65] An example of kidney disease that is characterized by an initial phase of glomerular hyperfiltration followed by progressive kidney function decline is diabetic nephropathy.[64,66] A similar reaction has been demonstrated in the non-diabetic 5/6-neprectomy rat model by Brenner *et al.*[65,67,68] In this respect, the merit of post-donation compensation of GFR above 50% of the pre-donation GFR for long-term outcomes has been questioned. So far, however, favorable long-term outcomes for living kidney donors point away from malignant hyperfiltration leading to adverse outcomes. Adaptation of GFR after surgical reduction of

nephrons in a healthy body might therefore be mechanistically different than adaptation for nephron loss due to systemic disease.[51,69] Conforming to this hypothesis, it has been suggested that the adaptive increase in GFR after unilateral nephrectomy is more likely to be the result of an increased renal plasma flow and/or increased filtration coefficient than an increased intraglomerular hydraulic pressure.[51,70] Possibly, a GFR increase due to increased renal plasma flow and/or increased filtration coefficient is driven by reserve capacity of the kidney and if so, its prognostic value for long-term kidney function might be different than the prognostic value of malignant hyperfiltration.

Even though it has been a subject of interest for almost a full century, still little is known about the mechanisms driving the kidney´s reserve capacity, let alone how to measure it. Physiological reserve capacity is defined as "the potential capacity of a cell, tissue or organ system to function beyond its basal level in response to alterations in physiologic demands"[71], as demonstrated in the kidney by maintaining GFR in response to loss of nephrons. The reserve capacity is one of the contributing factors to physical resilience, which is defined as "the ability to resist functional decline or recover functional health following a stressor".[71] Diminution of the reserve capacity is believed to result in frailty, a state in which less or no physical resilience remains. If the post-donation increase in single-kidney GFR is an expression of resilience, it would be highly relevant to identify potential living kidney donors who have reduced reserve capacity at donor evaluation, because this could result in less resilience after donation. Age and kidney size are the most well-established determinants of post-nephrectomy GFR compensation, with older age and smaller kidneys being associated with less post-donation compensation.[72–77] Pre-nephrectomy GFR has been shown to be positively associated to post-nephrectomy compensation in some studies and negatively in other studies, where it should be mentioned that definitions of post-nephrectomy compensation differ between studies.[72,76,78] Also, some studies describe hypertension, obesity and diabetes as determinants of less compensation.[73,75,79] These studies suggest that donors that carry risk factors for impaired kidney function at pre-donation exhibit less compensation post-donation, possibly because they had a diminished reserve capacity. Yet, the prognostic implication of less or more compensation for long-term kidney function remains unknown.

Thesis outline and aims

This thesis consists of three parts, in which each part focuses on kidney health assessment in a different time frame: pre-donation (**Part A**), at the time of donation (**Part B**) and post-donation (**Part C**).

Part A

The first part explores existing and new methods to assess glomerular function and health at donor evaluation. Studies in this part will address the optimal method to assess GFR in potential living kidney donors and the role of kidney biopsies for ruling out glomerular disease. First, we hypothesized that due to the underestimation of mGFR by eGFR, it could be that availability of mGFR in a transplant program allows acceptance of donors with lower eGFR, thereby possibly increasing the number of eligible kidney donors. We expected this effect to become visible by a lower mean eGFR in donor groups that were selected based on mGFR than in donors that were selected based on eGFR. Results of this study are described in **Chapter 2** of this thesis.

Because mGFR is rarely (routinely) available in transplant centers, we also investigated the capacity of eGFR to predict pre- and post-donation GFR in **Chapter 3** and **Chapter 4**. First, we studied which of the five available CKD-EPI equations (which are most widely used to estimate GFR) is most accurate for predicting pre- and post-donation GFR in **Chapter 3**. It has been shown that the CKD-EPI equations that include both serum creatinine as cystatin C are most accurate for estimating GFR in the general population. This has also been confirmed in cross-sectional analyses in living kidney donors. We hypothesized that combining cystatin C with creatinine to estimate pre-donation GFR would also improve prediction of post-donation GFR. Additionally, because serum creatinine is affected by muscle mass, we hypothesized that cystatin C-based CKD-EPI equations would perform particularly well in a subgroup of donors with low or high muscle mass. Because the CKD-EPI equations were not developed in living kidney donors, we also aimed to investigate whether prediction of post-donation GFR in living kidney donors could be improved with a new donor-specific equation based on pre-donation parameters in **Chapter 4**. We developed the equation in an internal development cohort and compared its performance to the creatinine-based CKD-EPI equation in an internal and external validation cohort.

Besides the GFR, there are more ways to assess glomerular health. For example, presence of protein/albumin or erythrocytes that do not derive from a urological problem are likely to derive from the glomerulus. Especially asymptomatic mi-

croscopic hematuria is a common finding in potential donors, since microscopic hematuria affects 8-21% of the general population.[80,81] Even though the prognosis of asymptomatic microscopic hematuria without other signs of kidney disease in the general population is excellent, living kidney donor guidelines advice to perform a kidney biopsy in potential kidney donors with microscopic hematuria (with no urological cause).[22,23] Because this is an invasive procedure that is not free of risk, and the risks of isolated microscopic hematuria in potential kidney donors are not clear, these biopsies are rarely performed in donors who are evaluated in many centers. In **Chapter 5**, we evaluated the long-term consequences of accepting these donors without performing a biopsy. We specifically studied the association between pre-donation microscopic hematuria and the post-donation course of eGFR, proteinuria and systolic blood pressure.

Part B

Although kidney biopsies are rarely performed at donor evaluation, a pre-implantation biopsy is always taken when the kidney is transplanted. The availability of these biopsies allowed us to study microstructural parameters in donor kidney biopsies and their associations with clinical characteristics and post-donation kidney function, which was the focus of **Part B** of this thesis. We investigated whether alterations in kidney tissue/subtle kidney damage, not captured by a reduced GFR, could provide additional prognostic information for post-donation kidney function outcomes in **Chapter 6** and **Chapter 7**. We studied microstructural kidney health by investigating associations of microstructural parameters including glomerular volume, tubular area and peritubular capillary density with each other and with pre- and post-donation GFR.

Part C

In the last part, **Part C**, we focused on post-donation kidney health by focusing on the increase in function of the remaining kidney. The aim of this part was to investigate whether a stronger increase in GFR was associated with better or worse long-term kidney function. We hypothesized that a stronger increase in single-kidney GFR could be an expression of more resilience and therefore might predict better long-term kidney function. In **Chapter 8**, we calculated the short-term increase in single-kidney GFR by subtracting 50% of the pre-donation GFR from the three months post-donation GFR. We investigated the predictive capacity of the short-term increase in single-kidney GFR for five and ten-year post-donation GFR and proteinuria. Additionally, we aimed to identify pre-donation determinants of the short-term increase in single-kidney GFR.

Because kidney donors are healthy individuals from who might be expected to have an intact reserve capacity and thus adequate compensatory response, we also aimed to study the prognostic value of the short-term post-nephrectomy increase in single-kidney GFR in a less healthy population. Therefore, in **Chapter 9**, we investigated the prognostic value of the short-term increase in single-kidney GFR for long-term kidney function in a cohort of patients that underwent unilateral nephrectomy for other reasons than kidney donation (mainly kidney cancer). These patients carried more comorbidities that might affect the kidney's reserve capacity. For this study, we collaborated with the Karolinska Institutet in Stockholm to use data from the Serum CREAtinine Measurements (SCREAM) project. The SCREAM project consists of the laboratory and healthcare use data of all inhabitants of the Stockholm region. We used procedure and diagnosis codes to identify all patients that underwent radical unilateral nephrectomy outside the setting of living kidney donation. The aim was to study whether the short-term increase in single-kidney GFR was associated with the risk of long-term progressive kidney function decline, which we defined as 30% decline in eGFR or initiation of kidney replacement therapy.

Overall, this thesis will cover aspects of kidney health assessment before, during, and after kidney donation, with potential implications for both living kidney donors and non-donors who undergo unilateral nephrectomy for other reasons.

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PART

A

2

CHAPTER

Impact of measured versus estimated glomerular filtration rate-based screening on living kidney donor characteristics: A study of multiple cohorts

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PLOSONE (2022)

ABSTRACT

Background

Most transplant centers in the Netherlands use estimated glomerular filtration rate (eGFR) for evaluation of potential living kidney donors. Whereas eGFR often underestimates GFR, especially in healthy donors, measured GFR (mGFR) allows more precise kidney function assessment, and therefore holds potential to increase the living donor pool. We hypothesized that mGFR-based donor screening leads to acceptance of donors with lower predonation eGFR than eGFR-based screening.

Methods

In this longitudinal cohort study, we compared eGFR (CKD-EPI) before donation in one center using mGFR-based screening (mGFR-cohort, n = 250) with two centers using eGFRbased screening (eGFR-cohort1, n = 466 and eGFR-cohort2, n = 160). We also compared differences in eGFR at five years after donation.

Results

Donor age was similar among the cohorts (mean±standard deviation (SD) mGFR-cohort 53 ±10 years, eGFR-cohort1 52±13 years, P = 0.16 vs. mGFR-cohort, and eGFR-cohort2 53±9 years, P = 0.61 vs. mGFR-cohort). Estimated GFR underestimated mGFR by 10±12 mL/ min/1.73m^2 (mean±SD), with more underestimation in younger donors. In the overall cohorts, mean±SD pre-donation eGFR was lower in the mGFR-cohort (91±13 mL/min/ 1.73m^2) than in eGFR-cohort1 (93±15 mL/min/1.73m^2 , P<0.05). However, these differences disappeared when focusing on more recent years, which can be explained by acceptance of more older donors with lower pre-donation eGFR over time in both eGFR-cohorts. Five years post-donation, mean±SD eGFR was similar among the centers (mGFR-cohort 62±12 mL/min/1.73m^2, eGFR-cohort1 61±14 mL/min/1.73m^2, eGFR-cohort2 62±11 mL/min/1.73m^2, P = 0.76 and 0.95 vs. mGFR-cohort respectively). In the mGFR-cohort, 38 (22%) donors were excluded from donation due to insufficient mGFR with mean±SD mGFR of 71±9 mL/min/1.73m^2.

Conclusions

Despite the known underestimation of mGFR by eGFR, we did not show that the routine use of mGFR in donor screening leads to inclusion of donors with a lower pre-donation eGFR. Therefore eGFR-based screening will be sufficient for the majority of the donors. Future studies should investigate whether there is a group (e.g. young donors with insufficient eGFR) that might benefit from confirmatory mGFR testing.

INTRODUCTION

Living kidney donor transplantation currently represents ~50% of the total kidney transplantations in the Netherlands [1]. The main goal of living kidney donor evaluation is to assess whether a donor is healthy enough to undergo surgery and maintain good health after the nephrectomy [2, 3]. An important part of screening consists of estimation and/or measurement of the glomerular filtration rate (GFR) before donation to determine whether the donor will retain sufficient kidney function after donation for life long safe kidney function. Glomerular filtration rate can easily be estimated (eGFR) by various equations based on serum creatinine or cystatin C, but the gold standard is assessment of the GFR by measuring the clearance of exogenous filtration markers (mGFR) [4]. The latter is expensive and laborious and therefore much less widespread in use in the Netherlands [5].

There is no consensus regarding the best method for kidney function assessment during the selection of living donors [2, 3, 6]. Some guidelines advise eGFR based on the chronic kidney disease epidemiology collaboration (CKD-EPI) equation, others advise use of 24h creatinine clearance or even mGFR. Due to cost and time advantages, most centers in the Netherlands estimate GFR based on creatinine clearance. The University Medical Center Groningen is the only center in the Netherlands that routinely performs mGFR measurements in every (potential) donor.

Even though mGFR is considered the gold standard, it is unclear whether its use is advantageous over the use of eGFR for living kidney donor screening. A well-known limitation in white populations of kidney function estimation equations based on serum creatinine is that in the higher ranges of GFR, true GFR is underestimated [7–13]. Consequently, donors with normal to high kidney function might be mistakenly classified as having insufficient kidney function when eGFR is used, possibly leading to exclusion from donation. This study aimed to compare pre- and post-donation eGFR of living kidney donors between two centers that base the decision to accept a donor based on eGFR and a center that uses mGFR for decision making. We hypothesized that mGFR-based screening allows acceptance of donors with lower mean pre-donation eGFR compared to the population from centers that use eGFR-based screening. In addition, post-donation safety was studied by comparing kidney function five years after donation in donors who have been evaluated using mGFR and eGFR.

MATERIALS AND METHODS

Study design

In this longitudinal cohort study in the Netherlands, we compared effective living kidney donors between one center that used mGFR-based donor evaluation (University Medical Center Groningen, mGFR-cohort) and two centers that used eGFR-based donor evaluation (eGFR-cohort1 = Erasmus MC, University Medical Center Rotterdam, and eGFRcohort2 = Radboud University Medical Center Nijmegen,). The study was approved by the institutional ethical review board of each participating center. For the mGFR-cohort, the study underwent ethical review in accordance with current ethical guidelines in 2014 as the TransplantLines biobank and cohort study (2014/077). The study was registered at clinicaltrials.gov under identifier NCT0327284 [14]. All donors included in the study signed informed consent for the use of their medical data for scientific research. In eGFR-cohort1 the study was approved by the EMC Medical Ethical Committee MEC-2019-0737. In eGFR-cohort1 and eGFR-cohort2, all donors have given written informed consent for the use of their medical data for scientific research. All procedures were conducted in accordance with the Declaration of Helsinki, Declaration of Istanbul, and the Dutch Scientific Guidelines.

Study population and measurements mGFR-cohort

In the University Medical Center Groningen, the selection criteria according to Dutch Living Kidney Donor guidelines (based on international guidelines) were used [3]. Instead of the recommended eGFR, mGFR was used to assess renal function before and after donation. A total of 1,113 potential living kidney donors were screened between 2006 and 2018 in Groningen. In this group, 977 donors were accepted for donation, of which 250 donated and had data for five-year follow-up available. The mGFR, measured as the urinary clearance of ^{125}I-iothalamate (S1 File), and eGFR (based on serum creatinine) were measured before donation in every (potential) donor and five years after donation. Measured GFR was corrected for body surface area (BSA, calculated according to Dubois et al.) [15]. Clinical decision making was based on pre-donation mGFR. Estimated GFR was retrospectively determined according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to enable comparison with the eGFR-cohorts and according to the Modification of Diet in Renal Disease (MDRD) equation and the Cockcroft-Gault (CG) equation for secondary analyses [12, 16, 17]. Twenty-fourhour urine samples were used to calculate the 24h creatinine clearance (CrCl). Besides kidney function measurements, clinical parameters such as weight, height, and blood pressure were measured during the visits.

Blood pressure was measured three times while seated with an interval of three minutes and a fourth time after standing straight for one minute using an automatic device as described previously [14].

Study population and measurements eGFR-cohort1

Between 1981 and 2019, 4,801 potential donors were screened for donation at the Erasmus MC, University Medical Center Rotterdam, the Netherlands. Of these donors, 2,144 donors eventually donated. For 647 donors, five-year follow-up was available. In order to enable comparison with the mGFR-cohort, donors that were screened before 2006 were excluded, rendering 466 donors eligible for this study. Glomerular filtration rate was assessed by equations based on serum creatinine, measured by enzymatic creatinine determination. In potential donors with unexpectedly low eGFR, 24-hour urine collection was performed to calculate endogenous creatinine clearance. When CrCl was adequate donation was allowed. Besides kidney function measurements, clinical parameters such as weight, height and blood pressure were measured during the visits.

Study population and measurements eGFR-cohort2

Between 2006 and 2014, 970 potential donors were screened for donation in the Radboud University Medical Center in Nijmegen, the Netherlands. Of these donors, 603 donors donated in these years. For 160 donors, five-year follow-up was available. Glomerular filtration rate was assessed by the equations based on serum creatinine and two 24-hour urine collection allowing calculation of the endogenous creatinine clearance. Serum creatinine was measured by enzymatic method. Besides kidney function measurements, clinical parameters such as weight, height and blood pressure were measured during the visits. The office blood pressure measurement was included in this study.

Statistical analyses

Data are presented as mean±standard deviation (SD) for normally distributed variables and as median (first quartile–third quartile) for non-normally distributed variables. The distribution was tested using histograms and probability plots. Binary variables are shown as 'number (%)'. Measured GFR data are reported as absolute values (mL/min) and corrected for body surface area according to Dubois et al. (mL/min/1.73m^2) [15]. To maintain consistency and enable comparison, eGFR was recalculated according to the CKD-EPI equation for all centers. Differences in characteristics of donors between the mGFR-cohort and eGFR-cohort1 and between the mGFR-cohort and eGFR-cohort2 were tested using the independent Student's t-test for normally distributed variables, the Mann-Whit-

ney U-test for non-normally distributed variables, and the chi-square test for proportions. To characterize donors with low pre-donation eGFR, we compared characteristics of 10% of donors with the lowest pre-donation eGFR to the other 90% of the donors using the tests mentioned above. Similarly, we compared donors with an underestimation of mGFR ≥10 and ≥20 mL/min/1.73m^2 by eGFR to donors with no underestimation or an underestimation <10 and <20 mL/min/1.73m^2, in order to identify donors at risk of underestimation by eGFR. Bias between pre- and post-donation eGFR and mGFR was calculated as the mean difference between both parameters. Because reason of exclusion from donation was mostly multifactorial and rarely solely dependent GFR, we did not analyze the number of donors excluded based on kidney function per center. SPSS version 23 for Windows (IBM, Armonk, NY) and Graphpad Prism 8 for Windows (Graphpad, San Diego, CA) were used to perform the analyses. P-values <0.05 were considered statistically significant.

RESULTS

Bias between eGFR and mGFR

The known underestimation of pre-donation mGFR by pre-donation eGFR (CKD-EPI) was also present in the mGFR-cohort (mean±SD bias = -10±12 mL/min/1.73m^2, **S1 Table**). This underestimation was visualized in a Bland-Altman plot (**Fig 1**). This bias became smaller five years after donation (-5±9 mL/min/1.73m^2). Pre-donation 24h CrCl overestimated pre-donation mGFR with a bias of 26±29 mL/min (**S2 Table**). Five years after donation, this overestimation was still present, although it was slightly reduced (18±19 mL/min).

Donors in whom pre-donation GFR was underestimated

The mGFR-cohort of donors was split into a group in which eGFR underestimated mGFR (≥10 mL/min/1.73m^2 difference) and a group in which eGFR did not underestimate mGFR (<10 mL/min/1.73m^2 difference), as shown in **Table 1**. Besides differences in kidney function, there were no statistically significant differences in clinical characteristics between donors in whom mGFR was underestimated by eGFR and donors in whom mGFR was not underestimated by eGFR. Donors in whom eGFR underestimated mGFR ≥20 mL/min/1.73m^2 were significantly younger than donors in whom the difference between eGFR and mGFR was <20 mL/min/1.73m^2 (mean±SD 50±8 vs. 54±10 years respectively, P = 0.02). A low eGFR compared to 24h CrCl was mainly limited to donors with higher height, weight, BMI and BSA (**S3 Table**). Difference between eGFR and

24h CrCl was more commonly <10 mL/min in female donors (**S3 Table**). An overestimation of mGFR by eGFR was present in 45 donors (**S4 Table**).

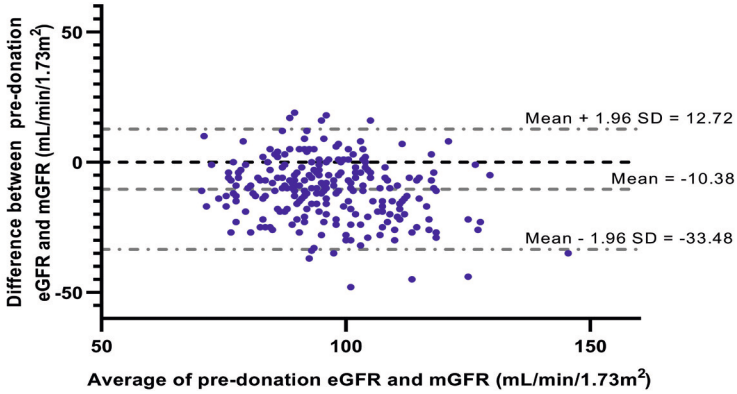

Figure 1. Bland-altman plot of pre-donation eGFR and pre-donation mGFR.

Bias between pre-donation eGFR and pre-donation mGFR is shown on the X-axis, the average between pre-donation eGFR and pre-donation mGFR is shown on the Y-axis. Mean±SD bias was -10.38 mL/min/1.73m^2, the 95% confidence interval of the mean bias was -33.48 to 12.72 mL/min/1.73m^2.

Table 1. Pre-donation characteristics of donors from the mGFR-cohort with an underestimation of mGFR$_{BSA}$ by eGFR ≥10 mL/min/1.73m^2.

	Underestimation ≥10 mL/min/1.73m2	Underestimation <10 mL/min/1.73m2	P value
Number, n (%)	121 (49)	127 (51)	-
CKD-EPI, mL/min/1.73m^2	88 ±13	94 ±12	<0.001
CrCl, mL/min	131 ±32	124 ±35	<0.001
mGFR, mL/min	122 ±24	109 ±19	<0.001
mGFR$_{/BSA}$, mL/min/1.73m^2	108 ±16	96 ±12	<0.001
Age, years	52 ±9	53 ±10	0.31
Sex, n (%) female	61 (50)	72 (57)	0.32
Race, n (%) Caucasian	121 (100)	127 (100)	-
Weight, kg	80 ±13	81 ±14	0.55
Height, cm	174 ±9	174 ±9	0.98
BMI, kg/m^2	26 ±3	27 ±4	0.38
BSA, m^2	1.95 ±0.20	1.96 ±0.20	0.70
SBP, mmHg	128 ±14	127 ±14	0.45
DBP, mmHg	77 ±9	76 ±9	0.48
Serum creat, µmol/L	78 ±13	70 ±12	<0.001

Table 1. Pre-donation characteristics of donors from the mGFR-cohort with an underestimation of $mGFR_{BSA}$ by eGFR ≥10 mL/min/1.73m². (continued)

	Underestimation ≥20 mL/min/1.73m2	Underestimation <20 mL/ min/1.73m2	P value
Number, n (%)	53 (21)	195 (79)	-
CKD-EPI, mL/min/1.73m²	89 ±14	92 ±12	0.20
CrCl, mL/min	139 ±28	124 ±34	0.01
mGFR, mL/min	133 ±23	110 ±20	<0.001
$mGFR_{/BSA}$, mL/min/1.73m²	116 ±15	98 ±12	<0.001
Age, years	50 ±8	54 ±10	0.02
Sex, n (%) female	23 (43)	110 (56)	0.09
Race, n (%) Caucasian	195 (100)	53 (100)	-
Weight, kg	82 ±13	81 ±14	0.46
Height, cm	176 ±10	174 ±9	0.28
BMI, kg/m²	26 ±3	26 ±4	0.99
BSA, m²	1.98 ±0.20	1.95 ±0.20	0.32
SBP, mmHg	127 ±14	128 ±14	0.62
DBP, mmHg	76 ±9	77 ±9	0.43
Serum creat, µmol/L	79 ±15	73 ±12	<0.001

Binary variables presented as n (%), continuous variables presented as mean ±SD
Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

Comparison of kidney function and clinical characterestics before donation

The characteristics of the living kidney donor populations before donation are shown in **Table 2**. Mean±SD age before donation was 53±10 (mGFR-cohort (Groningen)), 53±12 (eGFR-cohort1 (Rotterdam)), and 54±10 (eGFR-cohort2 (Nijmegen)) years and 54%, 54%, and 45%, respectively were female. Mean±SD eGFR (CKD-EPI) before donation was 91±13 mL/min/1.73m² in the mGFR-cohort, which was lower than in eGFR-cohort1 (93±15 mL/ min/1.73m², P = 0.20) and eGFR-cohort2 (94±12 mL/min/1.73m², P = 0.01) where eGFR formed the basis for screening. Distributions of pre-donation eGFR (CKD-EPI) for the different centers are shown in **Fig 2**. Mean±SD $mGFR_{/BSA}$ before donation was 101±15 mL/min/ 1.73m² in the mGFR-cohort. Pre-donation systolic blood pressure (SBP) was higher in eGFRcohort2 (137±16 mmHG) compared to the mGFR-cohort (128±14 mmHg, P<0.001) and slightly different between eGFR-cohort1 (130±16 mmHg) and the mGFR-cohort (P = 0.05). This difference

is probably explained by the use of office blood pressure in eGFR-cohort2. Body size measurements (height, weight, BMI and BSA) did not show major differences before and after donation between the cohorts.

Table 2. Characteristics of the living kidney donors during screening.

	mGFR-cohort	eGFR-cohort1	P vs. mGFR-cohort	eGFR-cohort2	P vs. mGFR-cohort
Number, n (%)	250	466	-	160	-
CKD-EPI, mL/min/1.73m^2	91 ±13	93 ±15	0.20	94 ±12	**0.02**
CrCl, mL/min	127 ±33	-	-	129 ±28	0.50
mGFR, mL/min	115 ±22	-	-	-	-
mGFR$_{/BSA}$, mL/min/1.73m^2	101 ±15	-	-	-	-
Age, years	53 ±10	53 ±12	0.91	54 ±10	0.41
Female sex, n (%)	134 (54)	252 (54)	0.90	72 (45)	0.89
Caucasian race, n (%)	250 (100)	450 (97)	-	160 (100)	-
Weight, kg	80 ±14	79 ±14	0.12	78 ±14	0.05
Height, cm	174 ±9	172 ±9	<0.001	173 ±8	0.13
BMI, kg/m^2	26 ±3	27 ±4	0.38	26 ±4	0.13
BSA, m^2	1.96 ±0.20	1.92 ±0.20	0.01	1.92 ±0.19	0.04
SBP, mmHg	128 ±14	130 ±16	0.05	137 ±16	<0.001
DBP, mmHg	76 ±9	78 ±9	0.07	81 ±8	<0.001
Use of antihypertensive medication, n (%)	43 (17)	79 (17)	0.93	24 (15)	0.56
Smoking, n (%)	59 (24)	-	-	52 (33)	0.05
Serum creat, µmol/L	74 ±13	74 ±14	0.62	72 ±12	0.18

Binary variables presented as n (%), continuous variables presented as mean ±SD
Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

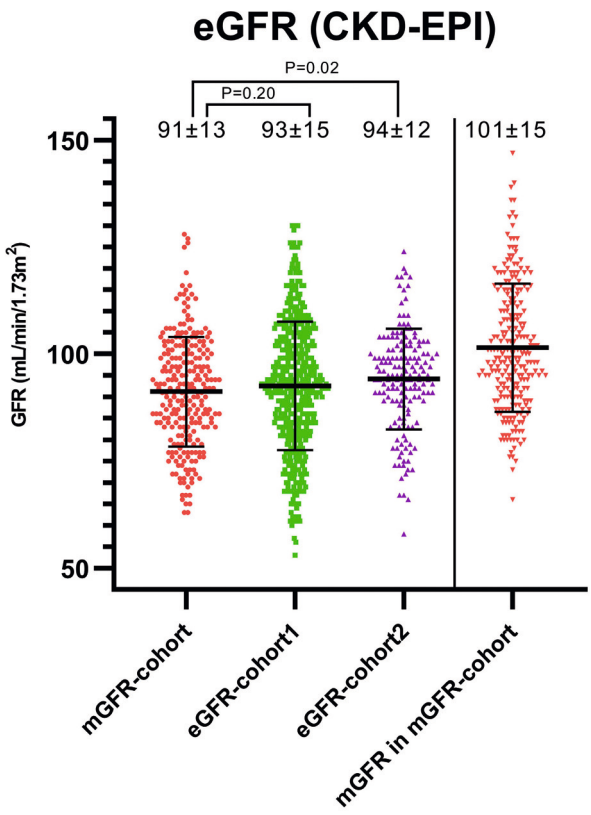

Figure 2. Distribution of pre-donation eGFR (CKD-EPI) per center.

Differences between mean pre-donation eGFR were tested using the independent sample T-test, P-values are shown in the Fig. Distribution of mGFR in the mGFRcohort was added on the right in the Fig.

Analysis of differences in eGFR over time

Because this study included donors that were screened during a large timeframe (especially in eGFR-cohort1), we performed secondary analyses to investigate whether the differences in pre-donation eGFR were consistent over time. We therefore split the cohort in two equal parts, which resulted in a group that was screened before 01-01-2009, and a group that was screened after 01-01-2009. **Fig 3** shows the distribution of pre-donation eGFR (CKD-EPI) before and after 2009 and shows that the differences that were seen in the total cohort, mainly depended on differences in pre-donation eGFR before 2009 (mean±SD eGFR in mGFRcohort: 90±12 mL/min/1.73m^2, eGFR-cohort1: 94±15 mL/min/1.73m^2, eGFR-cohort2: 97±11 mL/min/1.73m^2). When focusing on data after 2009, the differences in pre-donation eGFR seem to disappear (mGFR-cohort: 92±13 mL/

min/1.73m^2, eGFR-cohort1: 92±15 mL/min/1.73m^2 , eGFR-cohort2: 93±12 mL/min/1.73m^2). When looking at age before and after 2009 (**Fig 4**), our data show that both eGFR-cohort1 and eGFR-cohort2 accepted older donors after 2009 compared to before 2009, although only significant in eGFR-cohort2 (mean±SD age eGFR-cohort1: 52±12 years before and 53±13 years after 2009 (P = 0.16), eGFR-cohort2: 51 ±10 before and 55±9 after 2009 (P = 0.01)), whereas in mGFR-cohort there does not seem to be a difference in age over time (before 2009 53±9 years vs. after 2009 53±10 years, P = 0.99). Mean BMI did not differ before and after 2009 in the three centers (**S1 Fig**).

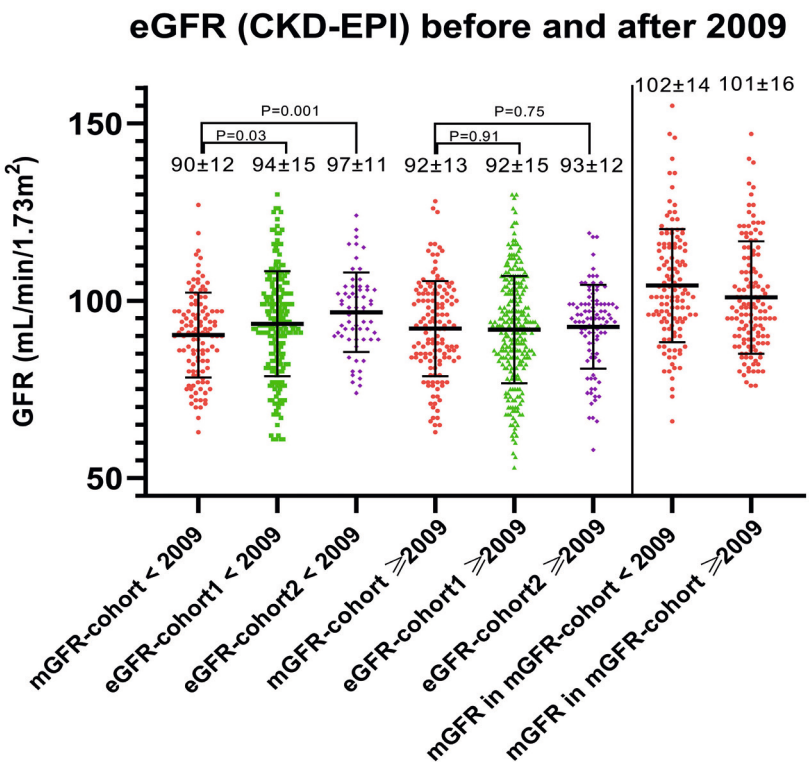

Figure 3. Distribution of pre-donation eGFR (CKD-EPI) before and after 2009 per center.

Differences between mean pre-donation eGFR were tested using the independent sample T-test, P-values are shown in the Fig. Distribution of mGFR in the mGFR-cohort was added on the right in the Fig.

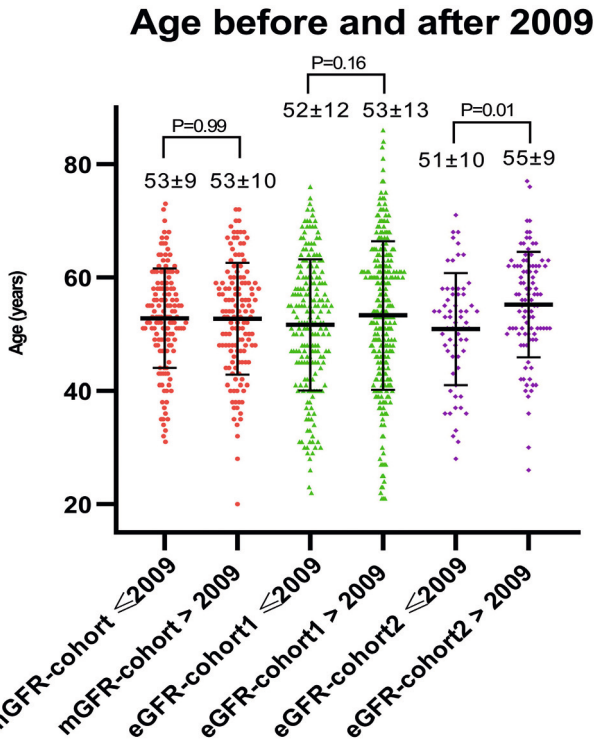

Figure 4. Distribution of age before and after 2009 per center.

Differences between mean age were tested using the independent sample T-test, P-values are shown in the Fig.

Living kidney donor characteristics five years after donation

Five years after donation, there was no difference in mean±SD eGFR (CKD-EPI) in the total cohort (mGFR-cohort: 62±12 mL/min/1.73m^2, eGFR-cohort1: 60±14 mL/min/1.73m^2 (P = 0.15 vs. mGFR-cohort), eGFR-cohort2: 61±11 mL/min/1.73m^2 (P = 0.65 vs. mGFRcohort, **Table 3** and **S2 Fig**). When looking at differences between the centers for the groups that were screened before 2009 and after 2009, we see no differences between the centers, but for all centers five-year post-donation eGFR was lower (mGFR-cohort: 64±12 mL/min/1.73m^2 before and 60±12 mL/min/1.73m^2 after 2009 (P = 0.01), eGFR-cohort1: 61±14 mL/min/ 1.73m^2 before and 59±13 mL/min/1.73m^2 after 2009 (P = 0.07), eGFR-cohort2: 63±11 mL/ min/1.73m^2 before and 60±11 mL/min/1.73m^2 after 2009 (P = 0.04), **S3 Fig**).

Table 3. Characteristics of the living kidney donors five years after donation.

	mGFR-cohort	eGFR-cohort1	P vs. mGFR-cohort	eGFR-cohort2	P vs. mGFR-cohort
Number, n	250	466	-	160	-
CKD-EPI, mL/min/1.73m^2	62 ±12	60 ±14	0.15	61 ±11	0.65
ΔCKD-EPI, mL/min/1.73m^2*	-29 ±10	-32 ±10	<0.001	-33 ±8	<0.001
CrCl, mL/min	85 ±22	-	-	-	-
mGFR, mL/min	76 ±16	-	-	-	-
mGFR$_{/BSA}$, mL/min/1.73m^2	67 ±11	-	-	-	-
Age, years	58 ±10	58 ±12	0.74	59 ±10	0.29
Weight, kg	83 ±15	81 ±15	0.17	80 ±16	0.15
BMI, kg/m^2	27 ±4	27 ±4	0.34	27 ±4	0.36
BSA, m^2	1.98 ±0.21	1.94 ±0.20	0.02	1.94 ±0.21	0.10
SBP, mmHg	127 ±14	133 ±16	<0.001	133 ±15	<0.001
DBP, mmHg	76 ±10	79 ±9	<0.001	79 ±7	0.01
Use of antihypertensive medication, n (%)	67 (27)	141 (30)	0.33	58 (36)	0.04
Smoking, n (%)	69 (28)	-	-	46 (29)	0.80
Serum creat, μmol/L	103 ±20	106 ±21	0.09	104±18	0.48

Binary variables presented as n (%), continuous variables presented as mean ±SD
*Calculated as: CKD-EPI 5 years after donation minus pre-donation CKD-EPI
Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

Secondary analyses of pre-donation kidney function

Mean±SD pre-donation 24-hour creatinine clearance (24h CrCl) was 127±33 mL/min in the mGFR-cohort and 129±28mL/min in eGFR-cohort2 (P = 0.50); eGFR-cohort1 did not routinely determine CrCl (**S4 Fig**). These results were similar before 2009 compared to after 2009. We also compared pre-donation eGFR according to the CG and MDRD equation before and after 2009, which yielded similar results to the CKD-EPI comparison (**S5** and **S6 Figs**).

Comparison of donors with marginal pre-donation eGFR

We subsequently focused on the 10% of donors with lowest pre-donation eGFR in the three cohorts (**Table 4**). In these donors from the mGFR-cohort, mean±SD pre-donation eGFR was 70±3 mL/min/1.73m^2 and mean±SD five-year post-donation eGFR was 48±6 mL/min/1.73m^2 (**Table 4**). Pre-donation mGFR$_{/}$

$_{BSA}$ was 86±9 mL/min/1.73m^2 and only decreased to 59±9 mL/min/1.73m^2 five years after donation. The 10% donors from eGFRcohort1 and eGFR-cohort2 with lowest pre-donation eGFR were older than the corresponding donors from the mGFR-cohort (65±9 years and 60±8 years respectively vs. 56±6 years (P<0.001 and P = 0.09 respectively)). Furthermore, BSA tended to be higher in these donors from the mGFR-cohort versus eGFR-cohort1 and eGFR-cohort2 (1.94±0.19 m^2 vs. 1.89±0.15 m^2 and 1.89±0.17 m^2 (P = 0.13 and P = 0.30, respectively), but power might be too limited to draw conclusions. The same applies to blood pressure (132±21 mmHg for the mGFR-cohort vs. 136±17 mmHg for eGFR-cohort1 and 138±22 mmHg for eGFRcohort2 (P = 0.34 and P = 0.52, respectively). In the mGFR-cohort, 5% of the donors had a pre-donation eGFR below the age-adapted threshold versus 3% in eGFR-cohort1 (P = 0.13) and 1% in eGFR-cohort2 (P = 0.04) (**S5 Table**). None of these donors had poor outcomes at five years after donation.

Table 4. Pre- and 5 year post-donation characteristics of 10% of the donors with lowest pre-donation eGFR per center.

	mGFR-cohort		eGFR-cohort1		eGFR-cohort2	
	Pre-donation	5 year post-donation	Pre-donation	5 year post-donation	Pre-donation	5 year post-donation
Number, n (%)	25	25	51	51	16	16
CKD-EPI, mL/min/1.73m^2	70 ±3	48 ±6	67 ±5	43 ±8	72 ±5	48 ±6
CrCl, mL/min	107 ±20	73 ±17	-	-	106 ±22	-
mGFR, mL/min	98 ±15	67 ±15	-	-	-	-
mGFR$_{/BSA}$, mL/min/1.73m^2	87 ±9	59 ±9	-	-	-	-
Age, years	56 ±6	62 ±7	65 ±9	71 ±9	60 ±8	66 ±8
Female sex, n (%)	15 (60)	15 (60)	29 (57)	29 (57)	9 (56)	9 (56)
Caucasian race, n (%)	25 (100)	25 (100)	51 (100)	51 (100)	16 (100)	16 (100)
Weight, kg	80 ±12	82 ±13	77 ±9	79 ±11	76 ±12	79 ±14
Height, cm	173 ±9	173 ±9	171 ±8	171 ±8	172 ±9	172 ±9
BMI, kg/m^2	27 ±3	27 ±3	26 ±3	27 ±3	26 ±3	27 ±3
BSA, m^2	1.94 ±0.19	1.97 ±0.19	1.89 ±0.15	1.90 ±0.16	1.89 ±0.17	1.91 ±0.22
SBP, mmHg	132 ±21	130 ±18	136 ±17	135 ±16	138 ±22	131 ±10
DBP, mmHg	79 ±9	77 ±13	79 ±8	77 ±10	81 ±7	78 ±5
Serum creat, µmol/L	90 ±12	121 ±21	90 ±12	127 ±22	87 ±11	119 ±18

Binary variables presented as n (%), continuous variables presented as mean ±SD

Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Donors that were excluded from donation in the mGFR-cohort

From 2006 to 2018, 173 potential donors were excluded from donation (**Table 5**). Mean±SD eGFR of these donors was 81±14 mL/min/1.73m^2 compared to 91±13 mL/min/1.73m^2 in the accepted group (P<0.001). In 16 of these donors, insufficient mGFR was the main reason for disapproval. In 20 donors, insufficient mGFR was one of multiple reasons for disapproval. In two donors, mGFR was considered too low for the recipient. The characteristics of the donors that were declined due to insufficient mGFR (N = 38) are also shown in Table 5. Mean±SD mGFR of these donors was 70±12 mL/min/1.73m^2 (P<0.001 vs. accepted donors). Female donors were more likely to be declined fordonation due to low GFR (84% female in the "declined due to GFR" group vs.54% female in the accepted group (P<0.001)). Declined donors were also significantly older with smaller body size measurements compared to accepted donors.

Table 5. Characteristics of "accepted", "declined" and "declined due to low mGFR" donors in the mGFR-cohort.

	Accepted*	Declined	P vs. accepted	Declined due to mGFR	P vs. accepted
Number, n	250	173	-	38	-
CKD-EPI, mL/min/1.73m^2	91 ±13	81 ±14	<0.001	70 ±12	<0.001
CrCl, mL/min	127 ±33	106 ±31	<0.001	77 ±23	<0.001
mGFR, mL/min	115 ±22	96 ±22	<0.001	72 ±8	<0.001
mGFR$_{/BSA}$, mL/min/1.73m^2	101 ±15	88 ±18	<0.001	71 ±9	<0.001
Age, years	53 ±10	60 ±11	<0.001	66 ±6	<0.001
Female sex, n (%)	134 (54)	100 (8)	0.39	32 (84)	<0.001
Caucasian race, n (%)	250 (100)	173 (100)	-	38 (100)	-
Weight, kg	80 ±14	78 ±14	0.06	69 ±9	<0.001
Height, cm	174 ±9	171 ±9	0.001	167 ±6	<0.001
BMI, kg/m^2	26 ±3	27 ±4	0.73	25 ±3	0.01
BSA, m^2	1.96 ±0.20	1.90 ±0.20	0.01	1.77 ±12	<0.001
SBP, mmHg	128 ±14	131 ±14	0.02	129 ±10	0.50
DBP, mmHg	76 ±9	77 ±10	0.90	76 ±9	0.89

Table 5. Characteristics of "accepted", "declined" and "declined due to low mGFR" donors in the mGFR-cohort. (continued)

	Accepted*	Declined	P vs. accepted	Declined due to mGFR	P vs. accepted
Use of antihypertensive medication, n (%)	43 (17)	46 (27)	0.02	11 (29)	0.08
Serum creat, µmol/L	74 ±13	79 ±13	<0.001	82 ±15	0.002

*Donors who were accepted, donated and had 5-year follow-up available
Binary variables presented as n (%), continuous variables presented as mean ±SD
Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

DISCUSSION

This study aimed to compare pre- and post-donation eGFR of living kidney donors between two centers that base the decision to accept a donor based on eGFR and a center that uses mGFR for decision making. We hypothesized that, due to systematic underestimation of mGFR by eGFR, mGFR-based screening allows acceptance of donors with lower pre-donation eGFR than a center that only uses eGFR. Findings confirm that pre-donation eGFR can indeed underestimate pre-donation mGFR, especially in younger donors. In the overall cohort, we found lower pre-donation eGFR in a center that uses mGFR for donor screening than in centers that use eGFR. However, when focusing on more recent data, these differences disappear, and therefore, routine use of mGFR for living kidney donor screening does not seem to add value compared to using eGFR on population level. Lastly, we did not find differences in fiveyear post-donation eGFR between centers that use eGFR- or mGFR-based donor screening.

Measuring the clearance of exogenous filtration markers is the best available method to assess GFR [18]. Because mGFR has cost and availability issues, eGFR equations are most widely used. In line with the literature, our results show an underestimation of mGFR by eGFR [7–13]. Mean pre-donation eGFR was lower in the mGFR-cohort, where clinical decision making was based on mGFR, than in centers that only used eGFR. However, when taking time into account, we saw that both eGFR-cohort1 and eGFR-cohort2 accepted donors with lower pre-donation eGFR after 2009 compared to before 2009, resulting in disappearance of the differences in pre-donation eGFR. A reasonable explanation for this is

that both centers accepted older donors after 2009 compared to before 2009, whereas in the mGFR-cohort there was no difference in eGFR and age before and after 2009. The increase in age and the consistency of BMI over time that we found in this study are consistent with previous results [19]. The introduction of a national living kidney donor guideline in the Netherlands in 2008, in which age-adapted thresholds for pre-donation eGFR were introduced might have contributed to more uniformity in donor selection policies resulting in more similarity in recent donor characteristics [3]. Our findings are in line with a previous study by Gaillard et al., who concluded that mGFR is the most efficient method for living donor screening, but when not available, age-adapted thresholds for eGFR are also convenient [20]. Furthermore, we did not find differences in five-year post-donation eGFR, despite differences in pre-donation GFR assessment methods, which further supports the impression that routine use of mGFR does not have an effect on mean eGFR on population level.

While routinely using mGFR in donor screening does not have an effect on the total population characteristics, we did find that in half of the donors from the mGFR-cohort, mGFR was underestimated by eGFR ≥10 mL/min/1.73m^2 and in 20% of the donors even >20 mL/ min/1.73m^2. Reasons for donor exclusion are mostly multifactorial and rarely solely based on insufficient kidney function. Still, kidney function plays a major role in the decision-making process of accepting a potential donor, and played an important role in the decision of 22% of the declined donors in the mGFR-cohort. If insufficient eGFR is a decisive factor in the decision to decline a potential donor, confirmative GFR assessment might be needed, especially in younger donors. This is supported by the finding that donors with the lowest 10% eGFR were younger in the mGFR-cohort (where mGFR was used) than in eGFR-cohort1 and eGFRcohort2 (where eGFR was used). Measuring creatinine clearance (CrCl) from 24-hour urine samples might be an alternative. However, besides the sampling errors that could cause measurement inaccuracy, 24h CrCl tends to overestimate mGFR [21]. This overestimation increases in the lower ranges of GFR, possibly due to an increased tubular secretion of creatinine, causing an increased error in donors with marginal kidney function. For the majority of potential donors, 24h CrCl combined with eGFR will be sufficient to assess kidney function, because the mGFR will likely be in between those values. However for borderline cases with for example a too low eGFR and acceptable 24h CrCl, it is dangerous to assume that the 24h CrCl will be closer to the mGFR value than the eGFR value. In such cases additional mGFR testing would be useful.

The current guidelines do not clearly specify how GFR should be assessed before living kidney donation [2, 3, 6]. This study supports the concept that assessment of mGFR is not needed in every donor, but could be considered for a selected group of potential donors, for example young donors with an insufficient eGFR, consistent with previous results [20]. The previously developed online calculator from Huang et al., that calculates the probability to reach a specific pre-donation mGFR threshold based on pre-donation eGFR, age, sex and race, could be a supportive tool to distinguish between donors who could and who likely do not benefit from confirmatory mGFR testing [22]. In our study, only age was associated with an underestimation of mGFR by eGFR >20 mL/min/1.73m^2, and we did not identify other characteristics that led to underestimation of mGFR. Future studies should focus on more detailed characterization of donors in whom eGFR is inaccurate.

Strengths of this study include the extensive renal function measurements with ^{125}I-Iothalamate in the mGFR-cohort. Furthermore, the comparisons were made in relatively large populations throughout the whole country with long-term follow-up. Also, consistent use of methods for kidney function determination in the centers limits confounding by indication. Yet, our study also has several limitations. First of all, the decision to accept a donor is multifactorial, and does not only rely on pre-donation GFR. Yet, we were able to identify 16 donors that were declined due to insufficient GFR and another 22 in whom GFR was one of multiple reasons for disapproval. Both estimated and measured GFR of these donors were lower than in the accepted donors. Data on declined donors in the other centers were not available. Lastly, the three populations mainly consisted of Caucasian donors. It is known that people of African ancestry (i.e. African Americans, Black U.K. people) on average have higher muscle mass, possibly leading to larger underestimation of GFR by the creatinine-based equations [23]. However, because end-stage kidney disease is more prevalent among African and African American ethnicities [23], extra caution might be needed when accepting donors from these ancestries with lower pre-donation eGFR. Recently, it has been suggested to remove the racial correction factors in the eGFR equations, which led to more underestimation of GFR in black individuals in the general population as compared to white individuals. How these equations affect the applicability of the results of the current study (i.e. in a population with higher GFR than the general population) remains to be investigated.

In conclusion, this study shows that routinely measuring GFR using exogenous filtration markers did not lead to a detectable difference in the donor population

compared to using eGFR. These results suggest that the routine use of mGFR does not seem to result in acceptance of donors with lower pre-donation eGFR on the population level, neither does it result in differences in five year post-donation eGFR. For the majority of potential donors eGFR and/or 24h CrCl may provide sufficient guidance. Future studies are needed to confirm our results and investigate whether a group could be identified (e.g. young donors) that might benefit from confirmatory mGFR testing.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Tessa Royaards for the excellent management of the Rotterdam database.

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SUPPLEMENTARY MATERIAL

S1 Methods. GFR measurement mGFR-cohort

GFR was calculated from measurements of the clearance of radiolabeled iothalamate (^{125}I-iothalamate).[1] Before constant infusion of iothalamate started, a blood sample was drawn from the donors. This blood sample was used for routine laboratory measurements. Subsequently, infusion of iothalamate at 0.04 ml/kg body weight was started. The infusion solution contained 0.04 MBq of ^{125}I-iothalamate (following an initial dose of 0.6 MBq ^{125}I-iothalamate) and 0.03 MBq ^{131}I-hippurate and was started at 8:00 a.m. at an infusion rate of 12 ml/h. After a stabilization period, measurements started at 10:00 a.m. Clearances were calculated as (U*V)/P and (I*V)/P, where U*V represents the urinary excretion, I*V represents the infusion rate of the tracer and P represents the plasma tracer concentration per clearance period. From clearance levels of these traces, GFR, effective renal plasma flow, and filtration fraction were calculated. Correction for incomplete bladder emptying and dead space was achieved by multiplying the urinary ^{125}I-Iothalamate clearances with plasma and urinary ^{131}I-hippurate clearance. The day-to-day variability of the mGFR is 2.5%.[2]

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Table S1. Pre- and five year post-donation bias between eGFR and $mGFR_{/BSA}$ in the mGFR-cohort

	Pre-donation	5 year post-donation
Mean bias	-10	-5
Standard deviation	12	9
Median bias	-10	-6
IQR	-19 to -2	-12 to 0
Range	-48 to 19	-34 to 20

Bias calculated as eGFR – $mGFR_{/BSA}$
Abbreviations: eGFR: estimated glomerular filtration rate; $mGFR_{/BSA}$: measured glomerular filtration rate corrected for BSA; BSA: body surface area; IQR: interquartile range.

Table S2. Pre- and post-donation bias between CrCl and $mGFR_{/BSA}$ in the mGFR-cohort

	Pre-donation	5y post-donation
Mean bias	26	18
SD	29	19
Median bias	23	16
IQR	7 to 44	6 to 29
Range	-50 to 128	-39 to 73

Bias calculated as CrCl – $mGFR_{/BSA}$
Abbreviations: CrCl: 24 hour creatinine clearance; $mGFR_{/BSA}$: measured glomerular filtration rate corrected for BSA; BSA: body surface area; SD: standard deviation; IQR: interquartile range.

Table S3. Pre-donation characteristics of donors from the mGFR-cohort and eGFR-cohorteGFR-cohort2 with an underestimation of CrCl by eGFR ≥10 mL/min.

	mGFR-cohort			eGFR-cohort2		
	Underestimation ≥10 mL/min	Underestimation <10 mL/min	P value	Underestimation ≥10 mL/min	Underestimation <10 mL/min	P value
Number, N (%)	188	36	-	139	21	-
CKD-EPI, mL/min/1.73m^2	90 ±13	93 ±11	0.21	94 ±12	95 ±10	0.63
CrCl, mL/min	135 ±30	86 ±15	<0.001	135 ±25	94 ±14	<0.001
mGFR, mL/min	117 ±22	102 ±16	<0.001	-	-	-
mGFR$_{/BSA}$, mL/min/1.73m^2	102 ±15	97 ±11	0.03	-	-	-
Age, years	52 ±9	56 ±12	0.06	53 ±10	55 ±7	0.37
Female sex, n (%)	93 (50)	28 (78)	0.002	55 (40)	17 (81)	<0.001
Caucasian race, n (%)	188 (100)	36 (100)	-	139 (100)	21 (100)	-
Weight, kg	83 ±13	71 ±11	<0.001	80 ±13	66 ±13	<0.001
Height, cm	175 ±9	168 ±9	<0.001	174 ±8	166 ±8	<0.001
BMI, kg/m^2	27 ±3	25 ±4	0.01	26 ±3	24 ±4	0.002
BSA, m^2	1.98 ±0.19	1.80 ±0.15	<0.001	1.94 ±0.18	1.73 ±0.17	<0.001
SBP, mmHg	128 ±14	129 ±15	0.73	138 ±15	135 ±22	0.56
DBP, mmHg	77 ±9	77 ±8	0.83	81 ±9	79 ±8	0.21
Serum creat, µmol/L	76 ±13	68 ±11	0.001	74 ±12	65 ±7	<0.001

Binary variables presented as n (%), continuous variables presented as mean ±SD

Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

Table S4. Pre-donation characteristics of donors from the mGFR-cohort with an overestimation of $mGFR_{BSA}$ by eGFR.

Number, N(%)	45
Overestimation	-6 ±5
CKD-EPI, mL/min/1.73m^2	98 ±10
CrCl, mL/min	122 ±28
mGFR, mL/min	105 ±18
$mGFR_{/BSA}$, mL/min/1.73m^2	92 ±10
Age, years	53 ±10
Female sex, n (%)	27 (60)
Caucasian race, n (%)	45 (100)
Weight, kg	82 ±14
Height, cm	174 ±10
BMI, kg/m^2	27 ±4
BSA, m^2	1.97 ±0.20
SBP, mmHg	125 ±14
DBP, mmHg	76 ±9
Serum creat, µmol/L	67 ±12

Binary variables presented as n (%), continuous variables presented as mean ±SD
Abbreviations: CKD-EPI: chronic kidney disease epidemiology collaboration equation; CrCl: creatinine clearance; mGFR: measured GFR; BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation.

Table S5. Number of donors with eGFR above and under age-adapted threshold according to the Dutch Living Kidney Donor Guidelines.

		mGFR-cohort		eGFR-cohort1		eGFR-cohort2	
Age category	**eGFR threshold**	**eGFR < threshold**	**eGFR ≥ threshold**	**eGFR < threshold**	**eGFR ≥ threshold**	**eGFR < threshold**	**eGFR ≥ threshold**
<40	86	3 (12%)	23 (88%)	4 (5%)	72 (95%)	0 (0%)	16 (100%)
40-49	77	4 (7%)	57 (93%)	7 (6%)	104 (94%)	1 (3%)	30 (97%)
50-59	68	5 (5%)	104 (95%)	2 (2%)	132 (98%)	0 (0%)	71 (100%)
60-69	59	0 (0%)	49 (100%)	0 (0%)	114 (100%)	1 (3%)	36 (97%)
>69	50	0 (0%)	5 (100%)	0 (0%)	31 (100%)	0 (0%)	5 (100%)
Total		**12 (5%)**	**238 (95%)**	**13 (3%)**	**453 (97%)**	**2 (1%)**	**158 (99%)**

Abbreviations: eGFR: estimated glomerular filtration rate.

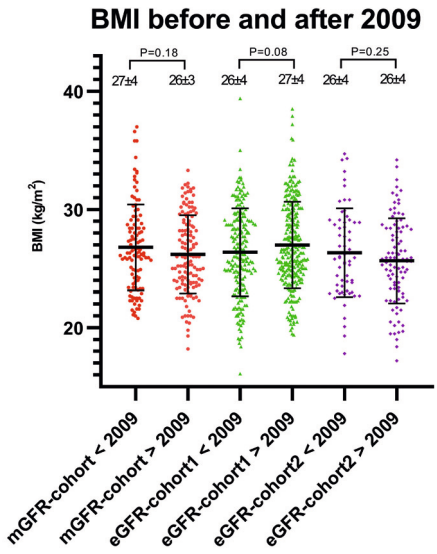

Figure S1. Distribution of pre-donation BMI before and after 2009 per center.

Differences between mean pre-donation BMI were tested using the independent sample T-test, P-values are shown in the figure.

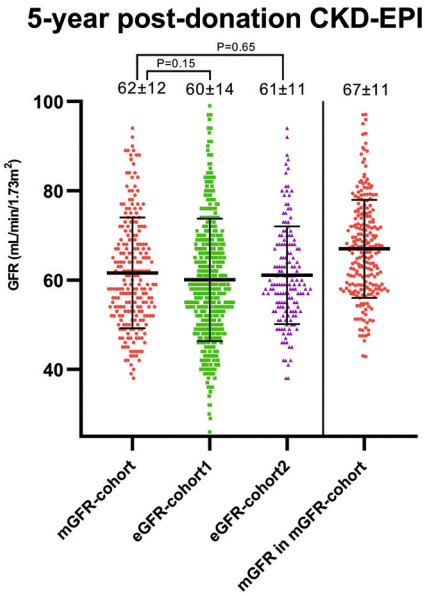

Figure S2. Distribution of five-year post-donation eGFR (CKD-EPI) per center.

Differences between mean five-year post-donation eGFR were tested using the independent sample T-test, P-values are shown in the figure. Five-year post-donation mGFR in the mGFR-cohort was added on the right in the figure.

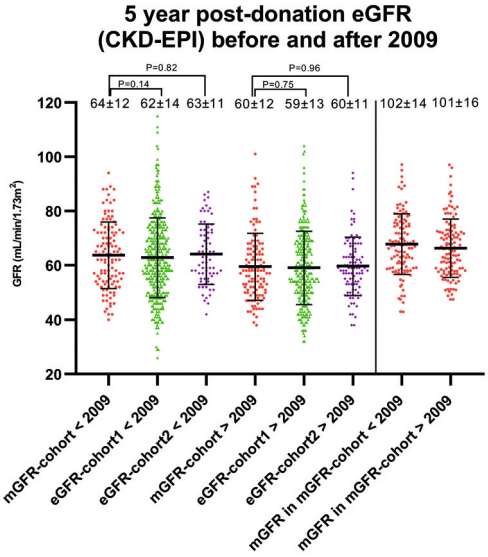

Figure S3. Distribution of five-year post-donation eGFR (CKD-EPI) before and after 2009 per center.

Differences between mean five-year post-donation eGFR were tested using the independent sample T-test, P-values are shown in the figure. Five-year post-donation mGFR before and after 2009 in the mGFR-cohort was added on the right in the figure.

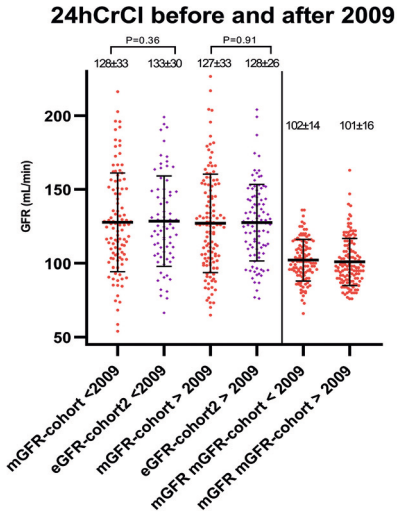

Figure S4. Distribution of pre-donation 24hCrCl before and after 2009 in the mGFR-cohort and eGFR-cohorteGFR-cohort2.

Differences between mean pre-donation 24hCrCl were tested using the independent sample T-test, P-values are shown in the figure. Pre-donation mGFR in the mGFR-cohort was added on the right in the figure.

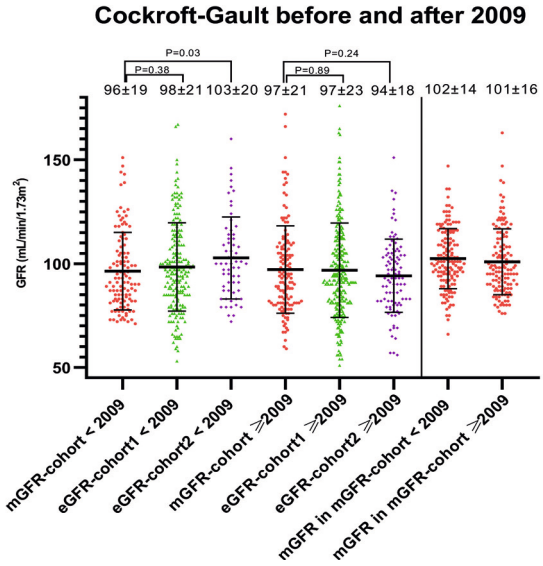

Figure S5. Distribution of pre-donation eGFR (CG) before and after 2009 per center.

Differences between mean pre-donation eGFR were tested using the independent sample T-test, P-values are shown in the figure. Pre-donation mGFR in the mGFR-cohort was added on the right in the figure.

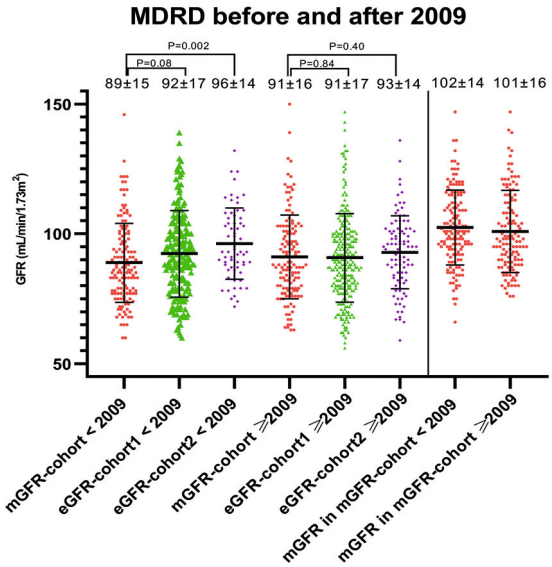

Figure S6. Distribution of pre-donation eGFR (MDRD) before and after 2009 per center.

Differences between mean pre-donation eGFR were tested using the independent sample T-test, P-values are shown in the figure. Pre-donation mGFR in the mGFR-cohort was added on the right in the figure.

3

CHAPTER

Pre-donation Assessment of Cystatin C to Improve Prediction of Pre- and Post-Donation GFR in Potential Living Kidney Donors

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REVISIONS FOR NEPHROLOGY DIALYSIS TRANSPLANTATION

ABSTRACT

Accurate estimation of glomerular filtration rate (GFR) is crucial in living kidney donation. While most eGFR equations are based on plasma creatinine, its levels are strongly influenced by muscle mass. Application of cystatin C (CysC)-based estimates before donation may improve both estimation of current GFR and prediction of post-donation GFR. We assessed the performance of CKD-EPI equations based on creatinine ($eGFR_{creat\text{-}2009}$, $eGFR_{creat\text{-}2021}$), cystatin C ($eGFR_{CysC\text{-}2012}$), or both ($eGFR_{combined\text{-}2012}$, $eGFR_{combined\text{-}2021}$) for estimating pre- and post-donation measured GFR in 236 living kidney donors. We subsequently focused on a subgroup of individuals with high/low muscle mass (25% highest/lowest 24-hour urinary creatinine excretion, sex-stratified and height-indexed). Pre-donation $eGFR_{combined\ 2012}$ and $eGFR_{combined\ 2021}$ showed the strongest associations with pre- and post-donation mGFR. Pre-donation $eGFR_{combined\ 2021}$ was most accurate for estimating both pre-donation (bias 0.5±12.8 mL/min/1.73m^2) and post-donation mGFR (bias 1.3±8.5 mL/min/1.73 m^2). In donors with high/low muscle mass, CysC-based equations (with or without creatinine) performed better compared to equations based on only creatinine. In conclusion, combined eGFR equations yielded a better estimate of pre- and post-donation mGFR, compared to estimates based on creatinine or CysC only. The added value of CysC is particularly pronounced in donors with high or low muscle mass.

INTRODUCTION

Assessment of kidney function plays an important role in the evaluation of potential living kidney donors, mainly to determine whether both the donor and the recipient will have sufficient kidney function after the donation or transplantation, respectively (1,2). So far, there has been no consensus – and thus no uniform policy – on how to assess pre-donation glomerular filtration rate (GFR) in potential donors (1,3–5). While determining GFR using exogenous filtration markers (measured GFR, mGFR) is the gold standard, it is not widely implemented due to financial and practical constraints. Estimating GFR using plasma creatinine-based estimation equations is easier and less costly, but drawbacks of this method include inaccuracy due to influences of non-GFR determinants, such as body composition and muscle mass (6,7). Moreover, previous studies concluded that relying on eGFR for the selection of living kidney donors results in unjustified exclusion of donors due to underestimation of kidney function (6,8–12). While it is important to accurately estimate pre-donation GFR in potential donors at time of evaluation, the goal is to assess whether sufficient kidney function will remain after donation. We recently developed an equation based on pre-donation plasma creatinine, age and sex to predict post-donation mGFR in living kidney donors (13). While the new equation outperformed plasma creatinine-based eGFR (CKD-EPI 2009), it still explained less than 40% of the variation in post-donation mGFR. In addition, this equation might perform worse in potential donors with muscle mass that deviates from average.

In the past decades, plasma cystatin C has been proposed as a promising marker to estimate GFR, as it is less dependent on body size and composition (7). Non-GFR determinants of plasma cystatin C include inflammation, diabetes and proteinuria (14–16), all of which are generally absent in potential living kidney donors. Addition of cystatin C improved accuracy and precision of the CKD-EPI equations, which has been confirmed in living kidney donors in cross-sectional analyses, both pre- and post-donation (17–20), yet its added value in pre-donation prediction of post-donation GFR remains unclear. Therefore, we investigated whether pre-donation addition of plasma cystatin C to creatinine-based eGFR equations improves the prediction of both pre- and post-donation GFR in a prospective cohort of living kidney donors with available data on iothalamate-measured GFR. We specifically investigated the added value of cystatin C in donors with low or high muscle mass.

METHODS

Study design and population

For this study, we used data from the ongoing, prospective TransplantLines Biobank and Cohort study (ClinicalTrials.gov identifier: NCT03272841), which aims to assess short- and long-term outcomes after solid organ transplantation and donation (21). For the current study, we selected 236 kidney donors enrolled in the TransplantLines study, with available pre-donation plasma creatinine, plasma cystatin C and pre- and post-donation mGFR (**Figure 1**). All patients were evaluated for donation between 2016 and 2021 at the University Medical Center Groningen in Groningen, The Netherlands. The study was approved by the institutional ethical review board (METc 2014/077). All procedures were conducted in accordance with the declaration of Helsinki and declaration of Istanbul.

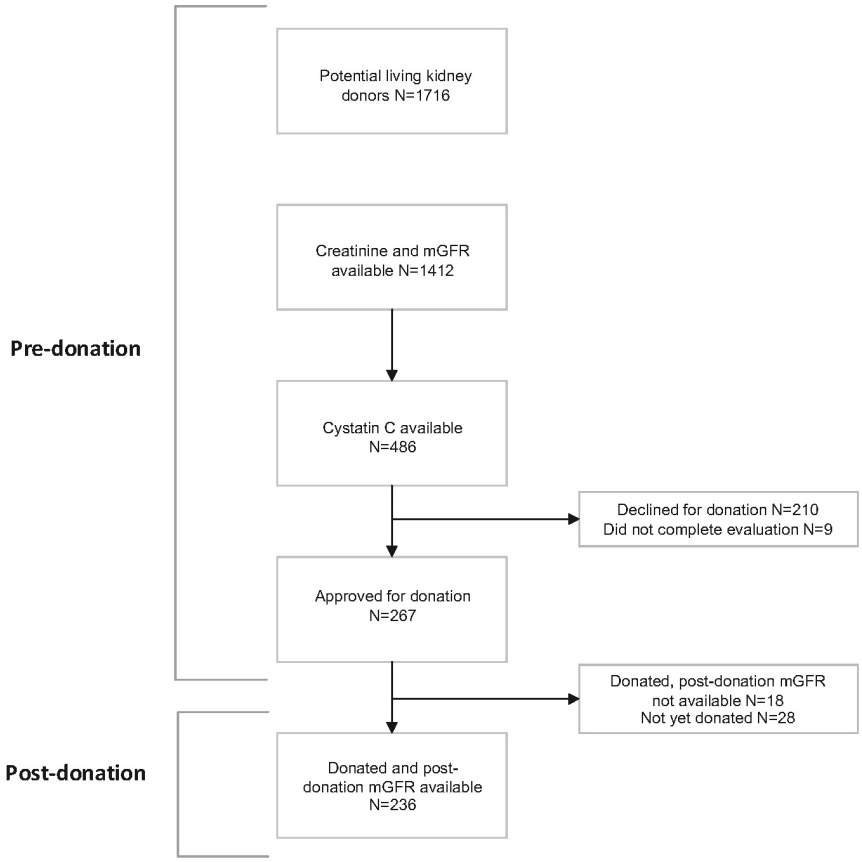

Figure 1. Overview of the study population. Abbreviations: mGFR = measured glomerular filtration rate

Measurement of plasma creatinine, plasma cystatin C, eGFR and mGFR
Plasma cystatin C concentrations were measured in EDTA plasma using validated particle-enhanced turbidimetric immunoassays (Gentian, Moss, Norway, for 198 patients and Roche diagnostics GmbH, Basel, Switzerland for 38 patients, respectively). Plasma creatinine was measured routinely in our central chemistry laboratory by an isotope dilution mass spectrometry (IDMS) traceable enzymatic assay on the Roche Modular (Roche Ltd., Basel, Switzerland). Estimated GFR was calculated according to CKD-EPI equations based on plasma creatinine ($eGFR_{creat\text{-}2009}$ and $eGFR_{creat\text{-}2021}$), cystatin C ($eGFR_{CysC\text{-}2012}$) and based on both markers combined ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) (7,22,23).

Measured GFR was determined using ^{125}I-iothalamate and ^{131}I-hippurate infusion as previously described (6). In short, ^{125}I-Iothalamate and ^{131}I-hippurate infusions were started and after a stabilization period, baseline measurements were performed in a steady state of plasma tracer levels. Clearances were calculated as (U*V)/P and (I*V)/P, where U*V represents the urinary excretion, I*V represents the infusion rate of the tracer and P represents the plasma tracer concentration per clearance period. We calculated mGFR from clearance levels of these tracers using (U*V)/P and corrected the renal clearance of ^{125}I-iothalamate for urine collection errors by multiplying the urinary ^{125}I-Iothalamate clearances with the ratio of plasma and urinary ^{131}I-hippurate clearance by using the following formula:

$$Corrected\ Clearance_{iot} = \frac{Clearance_{hip}(I\ \times V/P)}{Clearance_{hip}(U\ \times V/P)} \times Clearance_{iot}\ (U\ \times V/P)$$

Measured GFR was corrected for body surface area (BSA) in all analyses.

Statistical analyses
Data are reported as mean (standard deviation) for normally distributed variables and median [interquartile range, IQR] for skewed data. Binary variables are shown as “number (%)”. In primary analyses, we investigated the performance of the pre-donation CKD-EPI equations to predict pre- and post-donation mGFR. This was done by univariable linear regression analysis and by assessment of accuracy and precision. Accuracy and precision were determined by the R squared, bias, root mean squared error, interquartile range of the bias, and the percentage of predicted GFR within 30% and 10% of mGFR (P_{30} and P_{10}). For cross-sectional accuracy, we calculated the difference between pre-donation eGFR and pre-donation mGFR. For longitudinal accuracy, we calculated the predicted post-donation mGFR by multiplying pre-donation eGFR by 0.66, which was based on the

mean change in pre- to post-donation mGFR in our cohort (-34%). This change is in line with current literature on short-term compensation of the remaining kidney (24). The bias was then calculated as the difference between the predicted value of post-donation mGFR (0.66*pre-donation eGFR) and true mGFR.

In secondary analyses, we investigated the association of pre-donation plasma creatinine and cystatin C with pre- and post-donation mGFR in uni- and multivariable linear regression analyses while adjusting for age and sex. Main reason to do this was to investigate whether our previously developed prediction equation for post-donation mGFR based on pre-donation creatinine, age and sex could be improved by addition of cystatin C. Next, we selected a subgroup of donors with low or high muscle mass by calculating the 24-hour urinary creatinine excretion, which was indexed for height (25). Donors were assigned to the subgroup (N=118) if they were in the lowest (N=59) or highest quartile (N=59) of 24-hour creatinine excretion, stratified for sex. We repeated the univariable linear regression analyses of pre-donation CKD-EPI equations, plasma creatinine and cystatin C in this subgroup. In sensitivity analyses, we repeated the univariable linear regression analyses in subgroups according to cystatin c assay. Statistical analyses were performed in SPSS version 23 for Windows (IBM, Armonk, NY), R version 3.0.1 (CRAN, Vienna, Austria), and Graphpad Prism 6 for Windows (Graphpad, San Diego, CA). P-values of <0.05 were considered statistically significant.

RESULTS

Characteristics of the living kidney donor population

Pre-donation characteristics of the living kidney donor population are shown in **Table 1**. At pre-donation, age was 56±11 years, 51% of the donors were female, BMI was 26±4 kg/m^2, systolic blood pressure was 126±14 mmHg, plasma creatinine was 77±15 µmol/L, plasma cystatin C was 0.84±0.14 mg/L and mGFR was 95±14 mL/min/1.73m^2. All donors had HbA1c levels <53 mmol/mol (7%). Post-donation mGFR was 62±10 mL/min/1.73m^2.

Table 1. Pre-donation characteristics of the living kidney donor population.

Age, years	56±11
Female sex, n (%)	120 (51%)
Weight, kg	80±13
Height, cm	175±9
BMI, kg/m^2	26±4
BSA, m^2	1.95±0.19
Waist to hip-ratio	0.90±0.10
SBP, mmHg	126±14
Plasma creatinine, µmol/L	77±14
Plasma cystatin C, mg/L	0.86±0.14
HbA1c, mmol/mol	36±3
Albumin 24h urine, mg	9 [7 – 12]
mGFR, mL/min/1.73m^2	96±14
$eGFR_{creat\text{-}2009}$, mL/min/1.73m^2	89±14
$eGFR_{CysC\text{-}2012}$, mL/min/1.73m^2	94±16
$eGFR_{combined\text{-}2012}$, mL/min/1.73m^2	93±14
$eGFR_{creat\text{-}2021}$, mL/min/1.73m^2	91±13
$eGFR_{combined\text{-}2021}$, mL/min/1.73m^2	96±14

Normally distributed variables: mean±SD, not normally distributed variables: median [IQR], binary variables: N (%).
Abbreviations: BMI: body mass index; BSA: body surface area; creat: creatinine; CysC: cystatin C; mGFR: measured glomerular filtration rate; SBP: systolic blood pressure.

Primary analyses

Prediction of pre-donation mGFR with pre-donation eGFR

Associations of pre-donation eGFR with pre-donation mGFR for the total cohort are shown in **Table 2**. The $eGFR_{creat\text{-}2009}$ equation had a standardized beta (St.β) of 0.55 for the association with pre-donation mGFR. For the $eGFR_{CysC\text{-}2012}$ equation the St.β was 0.48 and when both markers were combined in the CKD-EPI 2012 and 2021 ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) equations the St.β were 0.59 and 0.57, respectively. When looking at the predictive capacity of pre-donation eGFR for pre-donation mGFR in **Table 3**, the bias of the $eGFR_{creat\text{-}2009}$ equation was -6.7 mL/min/1.73m^2, with a root mean squared error (RMSE) of 13.4 mL/min/1.73m^2. We also calculated the percentage of estimated GFR values that differed <30% and <10% from measured GFR (P_{30} and P_{10}, respectively). For $eGFR_{creat\text{-}2009}$, the P_{30} was 95% and the P_{10} was 50%. When cystatin C was used solely in the CKD-EPI 2012 equation ($eGFR_{CysC\text{-}2012}$), the bias decreased to -2.2

mL/min/1.73m^2 with an RMSE of 15.6 mL/min/1.73m^2, but P_{30} and P_{10} decreased to 89% and 44%, respectively. Combining creatinine with cystain C in the CKD-EPI 2012 equation ($eGFR_{combined\text{-}2012}$) resulted in a bias of -3.2 mL/min/1.73m^2, with an RMSE of 12.7 mL/min/1.73m^2 and a P_{30} and P_{10} of 97% and 56%, respectively. The update of the creatinine-based CKD-EPI 2009 equation ($eGFR_{creat\text{-}2009}$) to the creatinine-based CKD-EPI 2021 ($eGFR_{creat\text{-}2021}$) did not materially change accuracy and precision. The update of the CKD-EPI 2012 including both creatinine and cystatin C to the 2021 version ($eGFR_{combined\text{-}2021}$) resulted in the lowest bias of all equations (0.5 mL/min/1.73m^2). The RMSE and interquartile range of the bias of the $eGFR_{combined\text{-}2021}$ equation were comparable to the $eGFR_{combined\text{-}2012}$ equation. The $eGFR_{combined\text{-}2021}$ equation had a P_{30} of 98% and P_{10} of 53%, compared to 97% and 56%, respectively, for the $eGFR_{combined\text{-}2012}$ equation. Overall, the CKD-EPI equations that included both creatinine and cystatin C performed better than the CKD-EPI equations including either of these markers alone. Bland-Altman plots of the $eGFR_{creat\text{-}2009}$, $eGFR_{CysC\text{-}2012}$ and $eGFR_{combined\text{-}2021}$ are shown in **Figure S1**.

Table 2. Univariable linear regression analyses of pre-donation plasma creatinine/cystatin C and clinical characteristics with pre- and post-donation mGFR.

	Total cohort (N=236)*				Low/high muscle mass subgroup (N=118)					
	Pre-donation mGFR		Post-donation mGFR		Pre-donation mGFR			Post-donation mGFR		
	St.β	R^2	St.β	R^2	St.β	R^2	P	St.β	R^2	P
mGFR	-	-	0.76	0.58	-	-	-	0.77	0.59	<0.001
$eGFR_{combined\text{-}2021}$	0.57	0.32	0.60	0.36	0.61	0.36	<0.001	0.62	0.38	<0.001
$eGFR_{creat\text{-}2021}$	0.53	0.28	0.52	0.27	0.49	0.24	<0.001	0.47	0.22	<0.001
$eGFR_{combined\text{-}2012}$	0.59	0.34	0.63	0.39	0.62	0.38	<0.001	0.64	0.40	<0.001
$eGFR_{CysC\text{-}2012}$	0.48	0.23	0.53	0.28	0.56	0.31	<0.001	0.59	0.34	<0.001
$eGFR_{creat\text{-}2009}$	0.55	0.29	0.56	0.31	0.52	0.26	<0.001	0.51	0.25	<0.001
Plasma cystatin C	-0.40	0.16	-0.46	0.21	-0.48	0.22	<0.001	-0.52	0.27	<0.001
Plasma creatinine	-0.28	0.07	-0.31	0.09	-0.20	0.03	0.04	-0.24	0.05	0.01

*For both outcomes in the total cohort, P-values of all associations were <0.001.

Abbreviations: creat: creatinine; CI: confidence interval; CysC: cystatin C; mGFR: measured glomerular filtration rate

Table 3. Accuracy and precision of the CKD-EPI equations for pre- and post-donation mGFR.

	Accuracy and precision pre-donation eGFR for pre-donation mGFR*				
	$eGFR_{creat-2009}$	$eGFR_{CysC-2012}$	$eGFR_{combined-2012}$	$eGFR_{creat-2021}$	$eGFR_{combined-2021}$
R squared	0.29	0.23	0.34	0.28	0.32
Bias	-6.7	-2.2	-3.2	-4.4	0.5
RMSE	13.4	15.6	12.7	13.2	12.8
IQR bias	-14.9 to 1.7	-11.7 to 9.6	-12.2 to 4.8	-13.2 to 3.8	-8.9 to 9.0
P_{30}	95%	89%	97%	96%	98%
P_{10}	50%	44%	56%	52%	53%
	Accuracy and precision pre-donation eGFR for post-donation mGFR**				
R squared	0.31	0.28	0.39	0.27	0.36
Bias	-3.5	-0.5	-1.2	-2.0	1.3
RMSE	9.0	10.0	8.2	9.2	8.5
IQR bias	-9.1 to 2.1	-7.0 to 5.2	-6.1 to 4.1	-8.2 to 4.1	-4.0 to 6.6
P_{30}	95%	93%	97%	97%	96%
P_{10}	51%	49%	61%	50%	53%

*Bias calculated as eGFR minus mGFR: positive bias represents overestimation and negative bias represents underestimation.

**For calculation of the bias of pre-donation eGFR for post-donation mGFR, we first calculated the predicted post-donation mGFR value by multiplying pre-donation eGFR by 0.66. The bias was then calculated as the difference between predicted post-donation mGFR (0.66*pre-donation eGFR) and true mGFR: positive bias represents overestimation and negative bias represents underestimation.

Abbreviations: eGFR: estimated glomerular filtration rate; IQR: interquartile range; mGFR: measured glomerular filtration rate; P_{30} and P_{10}: percentage of bias within 30 or 10% of mGFR; RMSE: root mean squared error.

Prediction of post-donation mGFR with pre-donation eGFR

Associations of pre-donation eGFR with post-donation mGFR for the total cohort are shown in **Table 2** and **Figure S2**. The $eGFR_{creat-2009}$ equation had a standardized beta (St.β) of 0.56 for the association with post-donation mGFR. For the $eGFR_{CysC-2012}$ equation the St.β was 0.53 and when both markers were combined in the CKD-EPI 2012 and 2021 ($eGFR_{combined-2012}$ and $eGFR_{combined-2021}$) equations the St.β was 0.63 and 0.60, respectively. For the prediction of post-donation mGFR (**Table 3**), the bias was calculated as (pre-donation eGFR*0.66) – true post-donation mGFR. The $eGFR_{creat-2009}$ equation had a bias of -3.5 mL/min/1.73m^2 and an RMSE of 9.0 mL/min/1.73m^2 and the $eGFR_{CysC-2012}$ equation had a bias of -0.5 mL/min/1.73m^2 with an RMSE of 10.0 mL/min/1.73m^2. The combined $eGFR_{combined-2012}$ equation had a bias of -1.2 mL/min/1.73m^2 and an

RMSE of 8.2 mL/min/1.73m^2. The updated $eGFR_{creat\text{-}2021}$ equation had a bias of -2.0 mL/min/1.73m^2 with an RMSE of 9.2 mL/min/1.73m^2 and the combined $eGFR_{combined\text{-}2021}$ overestimated post-donation mGFR by 1.3 mL/min/1.73m^2 with an RMSE of 8.5 mL/min/1.73m^2. Although the $eGFR_{CysC\text{-}2012}$ equation seemed to have the lowest bias, this equation had the lowest P_{30} and P_{10} (93% and 49% respectively). The combined CKD-EPI 2012 and 2021 ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) equations had the highest P_{30} and P_{10} (2012: P_{30}=97%, P_{30}=61%, 2021: P_{30}=96%, P_{30}=53%).

Secondary analyses

Associations of pre-donation plasma cystatin C and creatinine with pre- and post-donation mGFR

We previously developed an equation that includes pre-donation creatinine, age and sex and predicts three months post-donation mGFR (13). To investigate whether this equation can be improved with addition of cystatin C, we also analyzed potential associations of pre-donation plasma creatinine and cystatin C with pre- and post-donation mGFR in uni- and multivariable linear regression models (**Table 2**, **Table S1** and **S2**). Pre-donation plasma cystatin C showed a stronger association with pre-donation mGFR than plasma creatinine (cystatin C: St.β=-0.40; creatinine: St.β=-0.28, **Table 2**). These results were similar for association of pre-donation cystatin C and creatinine with post-donation mGFR (cystatin C: St.β=-0.46; creatinine: St.β=-0.31, **Table 2**). Addition of pre-donation cystatin C to a multivariable linear regression model containing pre-donation creatinine, age and sex predicting pre-donation mGFR significantly improved the model R^2 from 0.32 to 0.37 (P<0.001, **Table S1**). When adding pre-donation cystatin C to a model including pre-donation creatinine, age and sex predicting post-donation mGFR the R^2 increased significantly from 0.32 to 0.40 (P<0.001, **Table S2**).

Donors with high or low muscle mass

We defined a subgroup that included donors with muscle mass in the lowest and highest quartile based on 24-hour creatinine excretion to study whether estimation of GFR improves with cystatin C in a group in whom plasma creatinine concentrations might be affected by muscle mass. Despite differences in sex and body size measurements, there were no statistically significant differences in mGFR or eGFR between the highest and lowest muscle mass quartile (**Table S3**). The two quartiles were combined after which the univariable linear regression analyses were repeated (**Table 2**). In this subgroup, the strength of

the association of creatinine (normally distributed) with both pre- and post-donation mGFR decreased (St.β=-0.20, P=0.04 (pre-donation mGFR); St.β=-0.24, P=0.01 (post-donation mGFR)), whereas the strength of the association between cystatin C and post-donation mGFR increased (St.β=-0.48, P<0.001 (pre-donation mGFR); St.β=-0.52, P<0.001 (post-donation mGFR)). The creatinine-based $eGFR_{creat\text{-}2009}$ and $eGFR_{creat\text{-}2021}$ equation showed the weakest correlations with pre-donation mGFR (2009: St.β=0.52, and 2021: St.β=0.49) and post-donation mGFR (2009: St.β=0.51 and 2021: St.β=0.47) compared to the other CKD-EPI equations. The $eGFR_{CysC\text{-}2012}$ equation had a St.β of 0.56 for pre-donation mGFR and a St.β of 0.59 for post-donation mGFR. Also in this subgroup, the CKD-EPI 2012 and 2021 ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) showed the strongest association with both pre-donation mGFR (2012: St.β=0.62 and 2021: St.β=0.61) and post-donation mGFR (2012: St.β=0.64 and 2021: St.β=0.62). Bland-Altman plots of cross-sectional performance of the $eGFR_{creat\text{-}2009}$, 2012 (CysC) and 2021 (creat + CysC) equations are shown in **Figure S3** and scatter plots of the longitudinal performance of the $eGFR_{creat\text{-}2009}$, 2012 (CysC) and 2021 (creat + CysC) equations are shown in **Figure S4**.

Sensitivity analyses

We performed a sensitivity analysis where we stratified the cohort according to the cystatin C assay that was used (Roche N=38, Gentian N=198). Characteristics of both groups are shown in **Table S4**. Repetition of the univariable linear regression analyses in these subgroups yielded similar results (**Table S5**).

DISCUSSION

This study aimed to investigate whether pre-donation cystatin C- (with or without creatinine-) based GFR estimation could improve assessment of pre- and post-donation GFR in living kidney donors. We found that the CKD-EPI 2012 and 2021 equations including both creatinine and cystatin C showed stronger associations with pre- and post-donation mGFR than CKD-EPI equations based on either creatinine or cystatin C alone. The pre-donation CKD-EPI 2012 and 2021 ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) were also most accurate and precise for pre- and post-donation mGFR. Addition of cystatin C to a multivariable linear regression model containing age, sex and plasma creatinine significantly increased the explained variance in pre- and post-donation mGFR. Improvements in associations with pre- and post-donation mGFR when cystatin C was used for pre-donation GFR estimation were particularly pronounced in subgroups of donors with high and low muscle mass. In this subgroup, plasma creatinine was

not associated with pre- or post-donation mGFR. Our study supports the added value of pre-donation cystatin C as a marker of pre- and post-donation kidney function in potential living kidney donors.

The KDIGO Living Kidney Donor Guideline (2017) recommends to confirm GFR using one or more of the following methods: measured GFR; measured creatinine clearance; estimated GFR ($eGFR_{combined\text{-}2012}$) and/or repeated estimated GFR from plasma creatinine (26). All these methods are different in terms of costs, feasibility and availability and also in terms of accuracy and precision, and therefore more clear guidance is needed. In the past decades, cystatin C has emerged as a promising marker of kidney function, being less dependent of body size and composition than creatinine (14). Addition of cystatin C to the CKD-EPI equation has been shown to improve accuracy and precision in cross-sectional analyses (7,17,23,27,28). Additionally, it has been shown recently that combining creatinine and cystatin C improves accuracy and precision of the European Kidney Function Consortium (EKFC) equation (29). In line, our study shows that addition of pre-donation cystatin C to a pre-donation creatinine-based multivariable linear regression model that was used to develop a prediction equation in a previous study by our group (13), improved the model fit for both pre- and post-donation mGFR. The pre-donation CKD-EPI 2012 and 2021 equations including both creatinine and cystatin C showed stronger associations with pre- and post-donation mGFR than the CKD-EPI equations that only included creatinine or cystatin C. In addition, the pre-donation CKD-EPI 2012 and 2021 ($eGFR_{combined\text{-}2012}$ and $eGFR_{combined\text{-}2021}$) showed better accuracy and precision for predicting pre- and post-donation mGFR. Future studies should investigate whether prediction of post-donation mGFR can be improved with donor specific cystatin C (with or without creatinine)-based donor equations or whether the existing CKD-EPI equations are sufficient.

Due to the effects of muscle mass and on plasma creatinine concentrations, plasma creatinine-based GFR assessment might not be accurate in individuals with muscle mass that deviates from average/the population the model was based on. Our subgroup analyses confirm these concerns. In donors with high or low muscle mass, the association of pre-donation creatinine with pre- and post-donation mGFR is not significant, while pre-donation cystatin C strongly associates with pre- and post-donation mGFR in this subgroup. This translates into stronger associations of CKD-EPI equations that include cystatin C than creatinine-based CKD-EPI equations with pre- and post-donation mGFR. This is in line with prior studies stating that the ratio between creatinine and cystatin

C is a useful predictor for sarcopenia (30–32). While many studies conclude that there is no association between cystatin C concentrations and muscle mass (33–35), Ivey-Miranda et al. found a significant association between muscle mass (assessed by creatinine excretion) and cystatin C in heart failure patients (36). However, as stated by the authors, the association was less strong than the association of creatinine with muscle mass and might be secondary to non-GFR determinants of cystatin C in this unhealthy population (36). Similarly, Macdonald et al. found a correlation between lean body mass and cystatin C, after adjusting for GFR, which they deemed logical since cystatin C is produced by all nucleated cells in the body including muscle cells (37). Cystatin C might therefore not be totally independent of muscle mass, but since it is not only produced by muscle cells, it might be superior to creatinine in patients with high or low muscle mass, which is supported by the results of our study. Other non-GFR determinants of plasma cystatin C that have been described include diabetes, CRP, white blood cell count and plasma albumin concentration (14), but the exact pathways though which these affect plasma concentrations of cystatin C are not fully understood. Hence, it is not clear when cystatin C-based eGFR should be interpreted with caution. It could be that these determinants are less variable or even absent in healthy individuals, making this a promising marker of kidney function in potential kidney donors, as also shown previously (38).

We found two prior studies that investigated the longitudinal association of cystatin C with the change in GFR from pre- to post-donation (39,40). Both studies found no advantages of cystatin C compared to plasma creatinine, but they were relatively small and did not use mGFR as reference method. In 2017, Bang et al. showed that pre-donation plasma cystatin C is a better marker of kidney function recovery after living kidney donation than eGFR determined by the MDRD equation (41). To our best knowledge, our study is the first to investigate the performance of pre-donation plasma cystatin C and the cystatin C-based CKD-EPI equations to assess absolute post-donation mGFR.

Guidelines agree that relying on creatinine based GFR assessment for selection of potential donors is insufficient for final decision making (5,42). In line, there is a group of donors in which post-donation mGFR is low, despite high pre-donation creatinine based eGFR (13), and at the same time, it has been shown that relying on plasma creatinine-based GFR assessment could lead to needless exclusion of potential donors due to underestimation of GFR (43,44). In the search for a proper alternative to the gold standard (mGFR) for donor evaluation that performs better than creatinine based eGFR, we think that our study favors the

estimation of GFR based on the combination of plasma creatinine and cystatin C. Comparable performance of the $eGFR_{combined\text{-}2021}$ and the $eGFR_{combined\text{-}2012}$ was in line with findings of Inker et al. in non-black individuals in the development study of the CKD-EPI 2021 equation (23). These results and the ethical concerns about the prior CKD-EPI equations may favor use of the race-free $eGFR_{combined\text{-}2021}$ equation. However, how our results apply to other non-white populations remains to be investigated. If doubt exists whether pre-donation GFR is sufficient, we suggest referral to a transplant center that has mGFR available.

Strengths of this study include the availability of both plasma creatinine and cystatin C as well as measured GFR. Moreover, availability of data on muscle mass enabled us to investigate the performance of cystatin C in donors with poor performance of creatinine. Yet, the study also has limitations. First, the sample size hampered investigating the predictive capacity (accuracy and precision) of the CKD-EPI equations in a validation data set. Second, our study consisted of only white donors, which may impact the performance of the CKD-EPI equations (7,22,23). Our results should therefore be validated in larger studies that also include non-white individuals.

In conclusion, we show that pre-donation GFR estimation based on the combination of creatinine and cystatin C improves prediction of pre- and post-donation mGFR compared to GFR estimation based on either of these markers alone. The added prognostic value of cystatin C was particularly pronounced in donors with high or low muscle mass.

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SUPPLEMENTARY MATERIAL

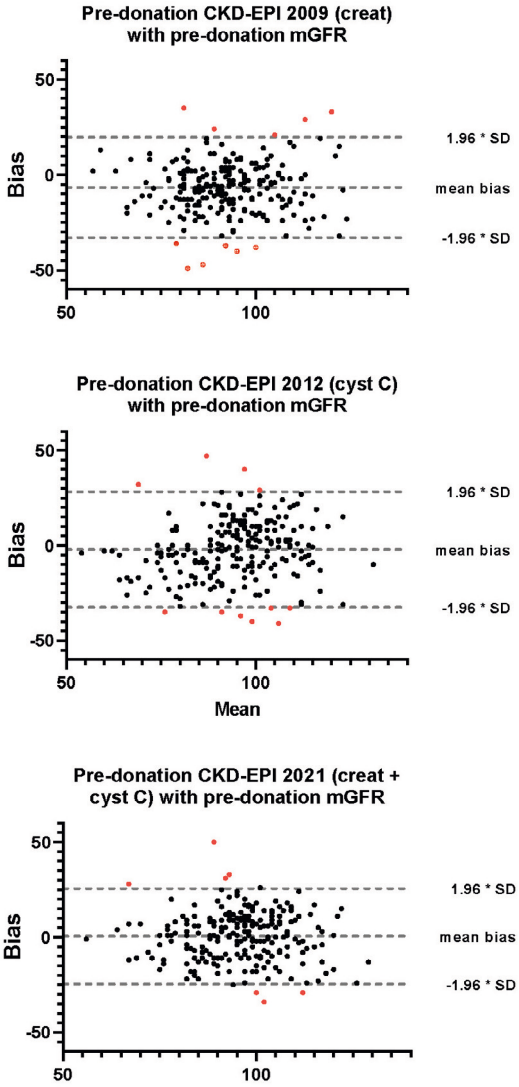

Figure S1. Bland-Altman plots of pre-donation CKD-EPI equations and pre-donation mGFR in the total cohort.

Upper = pre-donation $eGFR_{creat-2009}$ with pre-donation mGFR; middle = pre-donation $eGFR_{cysC-2012}$ with pre-donation mGFR; lower = pre-donation $eGFR_{combined-2021}$ with pre-donation mGFR. Bias calculated as eGFR minus mGFR: positive bias indicates overestimation and negative bias represents underestimation. Values outside the 95% confidence interval of the bias are displayed in red.

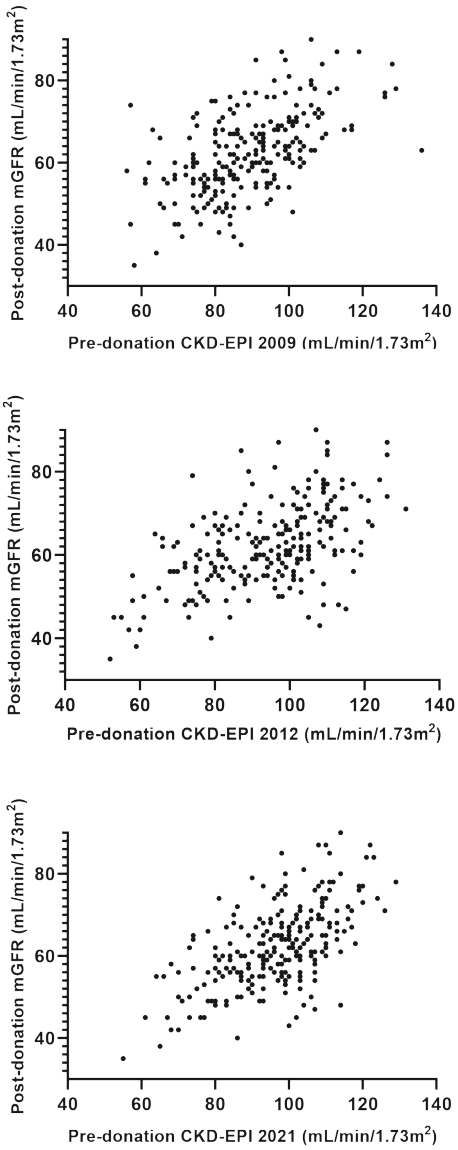

Figure S2. Scatter plots of pre-donation CKD-EPI equations and post-donation mGFR in the total cohort.

All figures: Y-axis = post-donation mGFR. Upper: X-axis = $eGFR_{creat-2009}$; middle: X-axis = $eGFR_{cysC-2012}$; lower: X-axis = $eGFR_{combined-2021}$.

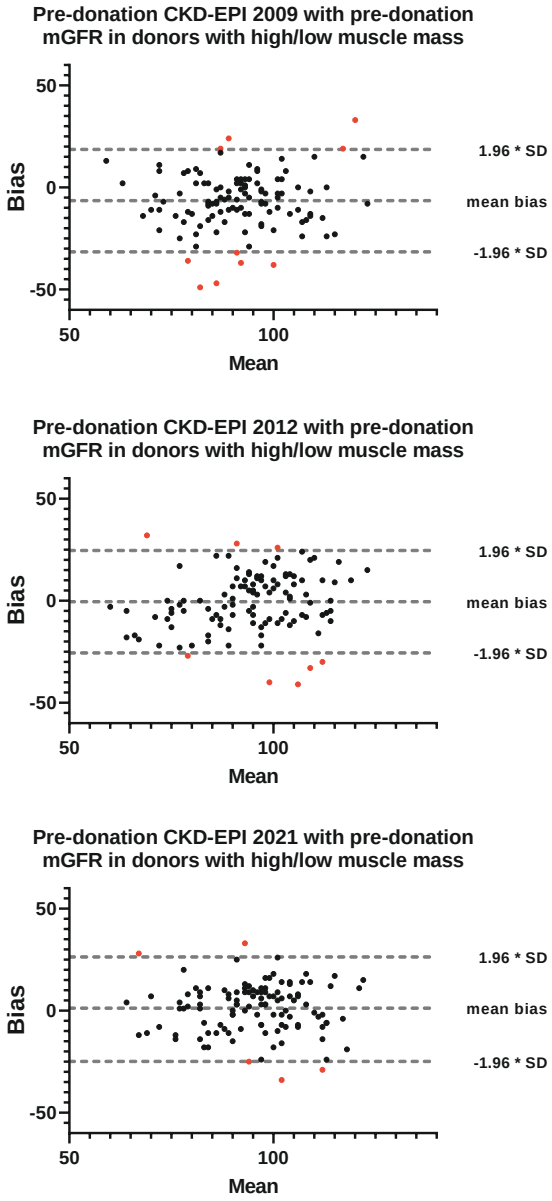

Figure S3. Bland-Altman plots of pre-donation CKD-EPI equations and pre-donation mGFR in donors with high/low muscle mass.

Upper = pre-donation $eGFR_{creat-2009}$ with pre-donation mGFR in donors with high or low muscle mass (N=118); middle = pre-donation $eGFR_{cysC-2012}$ with pre-donation mGFR in donors with high or low muscle mass (N=118); lower = pre-donation $eGFR_{combined-2021}$ with pre-donation mGFR in donors with high or low muscle mass (N=118). Bias calculated as eGFR minus mGFR: positive bias indicates overestimation and negative bias represents underestimation. Values outside the 95% confidence interval of the bias are displayed in red.

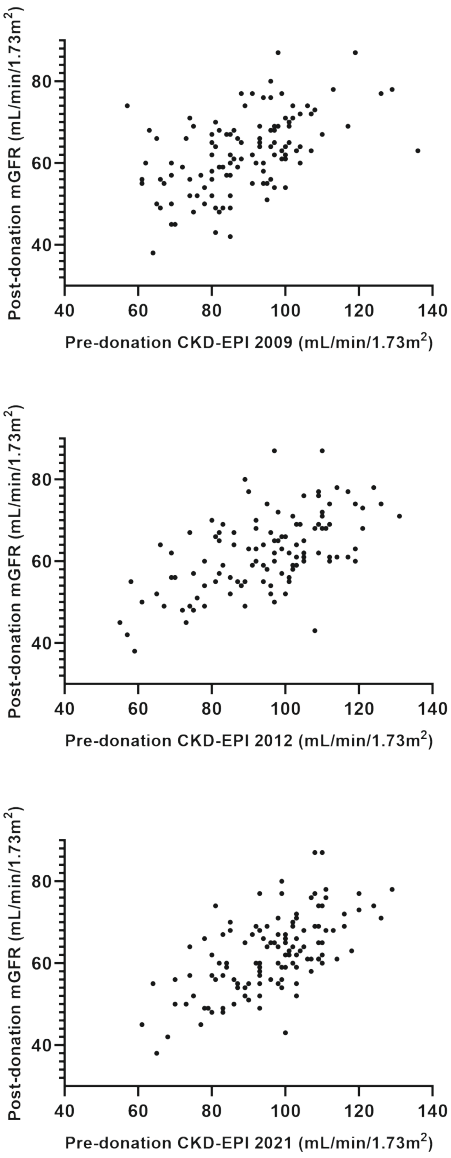

Figure S4. Scatter plots of pre-donation CKD-EPI equations and post-donation mGFR in donors with high/low muscle mass.

All figures: Y-axis = post-donation mGFR. Upper: X-axis = $eGFR_{creat-2009}$; Middle: X-axis = $eGFR_{cysC-2012}$; Lower: X-axis = $eGFR_{combined-2021}$.

Table S1. Multivariable linear regression models of the association between pre-donation serum creatinine/cystatin C with pre-donation mGFR.

	St.β	R^2	P	95% CI
Model 1				
Age	-0.41	0.32	<0.001	-0.52 to -0.30
Female sex	-0.38		<0.001	-0.52 to -0.25
Creatinine	-0.48		<0.001	-0.61 to -0.34
Model 2				
Age	-0.35	0.37	<0.001	-0.46 to -0.24
Female sex	-0.39		<0.001	-0.52 to -0.26
Creatinine	-0.38		<0.001	-0.52 to -0.24
Cystatin C	-0.26		<0.001	-0.37 to -0.14

R^2 change model 2 vs. model 1: P<0.001
Abbreviations: mGFR: measured glomerular filtration rate.

Table S2. Multivariable linear regression models of the association between pre-donation serum creatinine/cystatin C with post-donation mGFR.

	St.β	R^2	P	95% CI
Model 1				
Age	-0.44	0.32	<0.001	-0.57 to -0.30
Female sex	-0.26		<0.001	-0.39 to -0.12
Creatinine	-0.44		<0.001	-0.55 to -0.34
Model 2				
Age	-0.37	0.40	<0.001	-0.48 to -0.27
Female sex	-0.27		<0.001	-0.39 to -0.14
Creatinine	-0.32		<0.001	-0.45 to -0.19
Cystatin C	-0.31		<0.001	-0.43 to -0.20

R^2 change model 2 vs. model 1: P<0.001
Abbreviations: mGFR: measured glomerular filtration rate.

Table S3. Characteristics of subgroup with muscle mass (24 hour creatinine excretion) in lowest and highest quartile.

	Lowest quartile	Highest quartile
N	59	59
Age, years	58±11	52±11
Female sex, n (%)	30 (51%)	29 (49%)
Weight, kg	73±12	86±13
Height, cm	173±10	176±9
BMI, kg/m^2	24±3	28±3
BSA, m^2	1.87±0.18	2.03±0.19
Waist to hip-ratio	0.91±0.14	0.90±0.09
SBP, mmHg	128±16	125±15
Plasma creatinine, µmol/L	73±14	83±15
Plasma cystatin C, mg/L	0.87±0.13	0.84±0.15
Height-indexed 24 hour creatinine excretion, mmol/24h per meter	5.4 [4.7 to 6.5]	9.8 [7.8 to 10.6]
mGFR, mL/min/1.73m^2	91±13	99±13
$eGFR_{creat\text{-}2009}$, mL/min/1.73m^2	90±14	85±15
$eGFR_{cysC\text{-}2012}$, mL/min/1.73m^2	92±15	97±18
$eGFR_{combined\text{-}2012}$, mL/min/1.73m^2	92±13	93±16
$eGFR_{creat\text{-}2021}$, mL/min/1.73m^2	94±13	87±14
$eGFR_{combined\text{-}2021}$, mL/min/1.73m^2	96±12	96±15

Normally distributed variables: mean±SD, not normally distributed variables: median [IQR], binary variables: N (%).
Abbreviations: BMI: body mass index; BSA: body surface area; creat: creatinine; cysC: cystatin C; mGFR: measured glomerular filtration rate; SBP: systolic blood pressure.

Table S4. Characteristics of subgroups according to cystatin C assay.

	Roche	Gentian
N	38	198
Age, years	57±12	56±11
Female sex, n (%)	22 (58%)	98 (50%)
Weight, kg	76±9	81±14
Height, cm	173±9	175±9
BMI, kg/m^2	26±3	26±4
BSA, m^2	1.90±0.14	1.96±0.19
Waist to hip-ratio	0.91±0.08	0.90±0.10
SBP, mmHg	125±13	126±14

Table S4. Characteristics of subgroups according to cystatin C assay. (continued)

	Roche	Gentian
Plasma creatinine, µmol/L	72±9	77±15
Plasma cystatin C, mg/L	0.95±0.12	0.84±0.14
mGFR, mL/min/1.73m^2	97±15	95±14
eGFR$_{creat-2009}$, mL/min/1.73m^2	88±12	89±14
eGFR$_{cysC-2012}$, mL/min/1.73m^2	83±14	96±16
eGFR$_{combined-2012}$, mL/min/1.73m^2	86±11	94±14
eGFR$_{creat-2021}$, mL/min/1.73m^2	93±11	91±13
eGFR$_{combined-2021}$, mL/min/1.73m^2	91±12	97±14

Normally distributed variables: mean±SD, not normally distributed variables: median [IQR], binary variables: N (%).
Abbreviations: BMI: body mass index; BSA: body surface area; creat: creatinine; cysC: cystatin C; mGFR: measured glomerular filtration rate; SBP: systolic blood pressure.

Table S5. Univariable linear regression analyses of pre-donation serum creatinine/cystatin C and clinical characteristics with pre-donation mGFR in subgroups according to cystatin C assay.

	Roche			Gentian		
	St.β	R^2	P	St.β	R^2	P
mGFR	-	-	-	-	-	-
eGFR$_{combined-2021}$	0.59	0.32	<0.001	0.60	0.35	<0.001
eGFR$_{creat-2021}$	0.45	0.18	0.01	0.55	0.30	<0.001
eGFR$_{combined-2012}$	0.60	0.34	<0.001	0.62	0.38	<0.001
eGFR$_{cysC-2012}$	0.63	0.38	<0.001	0.54	0.30	0.001
eGFR$_{creat-2009}$	0.46	0.19	0.003	0.57	0.32	<0.001
Plasma cystatin C	-0.49	0.22	<0.001	-0.43	0.18	<0.001
Plasma creatinine	-0.19	0.01	0.26	-0.29	0.08	<0.001

Abbreviations: creat: creatinine; CI: confidence interval; cysC: cystatin C; mGFR: measured glomerular filtration rate.

4

CHAPTER

Prediction of measured GFR after living kidney donation from pre-donation parameters

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NEPHROLOGY DIALYSIS TRANSPLANTATION (2023)

ABSTRACT

Background

One of the challenges in living kidney donor screening is to estimate remaining kidney function after donation. Here we developed a new model to predict post-donation measured glomerular filtration rate (mGFR) from pre-donation serum creatinine, age and sex.

Methods

In the prospective development cohort (TransplantLines, n = 511), several prediction models were constructed and tested for accuracy, precision and predictive capacity for short- and long-term post-donation ^{125}I-iothalamate mGFR. The model with optimal performance was further tested in specific high-risk subgroups (pre-donation eGFR <90 mL/min/1.73 m^2, a declining 5-year post-donation mGFR slope or age >65 years) and validated in internal (n = 509) and external (Mayo Clinic, n = 1087) cohorts.

Results

In the development cohort, pre-donation estimated GFR (eGFR) was 86 ± 14 mL/min/1.73 m^2 and post-donation mGFR was 64 ± 11 mL/min/1.73 m^2. Donors with a pre-donation eGFR ≥90 mL/min/1.73 m^2 (present in 43%) had a mean post-donation mGFR of 69 ± 10 mL/min/1.73 m^2 and 5% of these donors reached an mGFR <55 mL/min/1.73 m^2. A model using pre-donation serum creatinine, age and sex performed optimally, predicting mGFR with good accuracy (mean bias 2.56 mL/min/1.73 m^2, R^2 = 0.29, root mean square error = 11.61) and precision [bias interquartile range (IQR) 14 mL/min/1.73 m^2] in the external validation cohort. This model also performed well in donors with pre-donation eGFR <90 mL/min/1.73 m^2 [bias 0.35 mL/min/1.73 m^2 (IQR 10)], in donors with a negative post-donation mGFR slope [bias 4.75 mL/min/1.73 m^2 (IQR 13)] and in donors >65 years of age [bias 0.003 mL/min/1.73 m^2 (IQR 9)].

Conclusions

We developed a novel post-donation mGFR prediction model based on pre-donation serum creatinine, age and sex.

INTRODUCTION

Kidney transplantation is the preferred treatment for most end-stage kidney disease (ESKD) patients, and living donors increasingly contribute to many kidney transplantation programs [1, 2]. In recent years, living kidney donor selection practices have been liberalized to compensate for donor shortages [3]. At the same time, it remains essential to optimally assess pre-donation kidney function and the impact of kidney donation on long-term kidney function in living donors. Estimated glomerular filtration rate (eGFR) equations, most widely used to assess kidney function, are imprecise to assess the true or measured GFR (mGFR) in the higher range and in donor candidates [4, 5]. Measuring the clearance of exogenous filtration markers such as iothalamate or iohexol is more precise, but also technically challenging, expensive, time-consuming and not widely available [4, 6]. Current guidelines for living kidney donation advise to accept healthy donors with an eGFR ≥90 mL/min/1.73 m^2 and, if eGFR is lower, to individualize the decision based on demographic and health profiles in relation to the transplant program›s threshold for acceptable risk [7]. However, little guidance is available on how to assess individualized risks.

The need of more precise, but simple, GFR evaluation tests that optimize the selection process has been underlined recently [8]. Some studies have attempted to address this issue by providing age-calibrated pre-donation GFR thresholds [9], but so far the only avaliable data suggest pre-donation eGFR thresholds based on cross-sectional analyses of mGFR in healthy non-donors, i.e. without taking the effect of donor nephrectomy into account [10, 11], and no studies have addressed the predictive value of pre-donation eGFR for post-donation mGFR. The average post-donation GFR is 66–70% of its pre-donation value [12], but it is unclear if pre-donation eGFR can be used at all to predict post-donation mGFR. Therefore, in the current study, we demonstrated which post-donation values were achieved for different pre-donation eGFR thresholds and used pre-donation serum creatinine and other widely available parameters to develop a predictive model for short-term post-donation mGFR. The performance of the model was validated in two independent cohorts of living kidney donors. We also tested the long-term performance of the predictive model, the performance in donors with a pre-donation eGFR <90 mL/min/1.73 m^2 and the performance in donors with negative post-donation mGFR slope.

MATERIALS AND METHODS

Study design and population (development and internal validation cohorts)

An overview of the design of our study is provided in Fig. 1. In this prospective cohort study, we performed mGFR measurements in 1020 living kidney donors who donated between 1984 and 2018 in the University Medical Center Groningen (UMCG), Groningen, The Netherlands. For the development cohort and internal validation cohort, we used data from the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov identifier: NCT03272841) at the UMCG. This is an observational study that aims to provide a better understanding of the causes of disease-related and ageing-related outcomes and health problems, both physical and psychological, in solid organ transplant recipients and donors. All participants gave written informed consent on enrollment. A detailed description of the study design and inclusion and exclusion criteria has been described previously [8]. We measured mGFR at 4 months before donation and at 3 months after living kidney donation, all as part of the screening program and post-donation evaluation. Donors were randomly assigned to a development cohort (n = 511) and an internal validation cohort (n = 509) for analysis of pre-donation parameters and post-donation mGFR. A subgroup of 409 donors had mGFR measurements at 5 years post-donation available and a subgroup of 110 donors at 10 years post-donation. The study was approved by the institutional ethical review board (METc 2014/077). All procedures were conducted in accordance with the Declaration of Helsinki and the Declaration of Istanbul.

As a result of our donor selection criteria, all donors were normotensive or had an adequately regulated blood pressure while taking no more than two antihypertensive drugs. Individuals with a history of diabetes (or an abnormal glucose tolerance test), kidney disease or cardiovascular events were not allowed to donate. Any other condition that was considered a potential threat to long-term renal or cardiovascular outcome was considered a contraindication for donation, at the discretion of the nephrologist involved in the selection procedure. Acceptance policies of living kidney donors in the UMCG are based on the Dutch Living Kidney Donor Guidelines [13], which are based on international guidelines [7, 14].

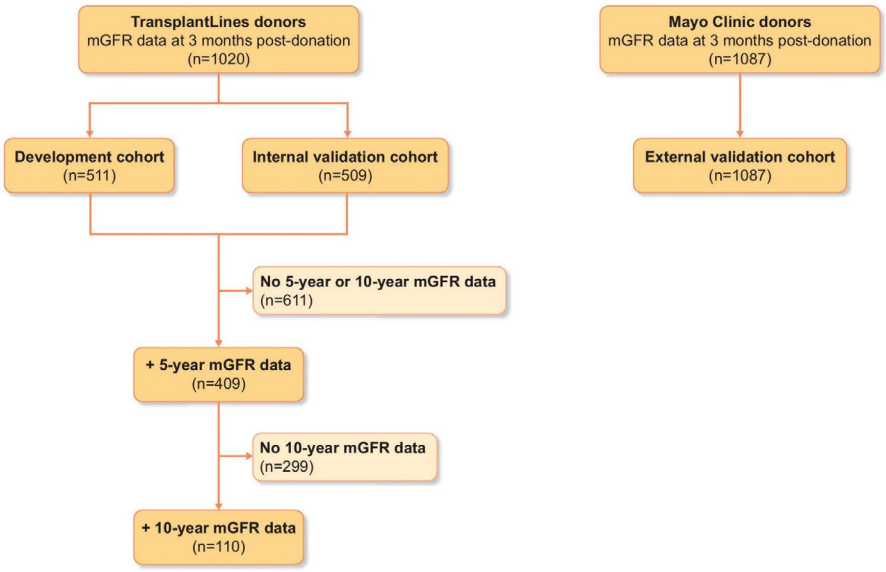

Figure 1. Overview of the study design and distribution of study participants.

Laboratory measurements

Serum creatinine was measured routinely in our central chemistry laboratory by an isotope dilution mass spectrometry (IDMS)-traceable enzymatic assay on the Roche Modular analyser (Roche, Mannheim, Germany) from 1 March 2006 onwards. Before this date, samples were measured by Jaffe alkaline picrate assay on the Merck Mega Analyzer (Merck, Darmstadt, Germany). Values obtained by the Jaffe method were converted to allow comparison with the Roche method by the formula ($Y^{\text{Roche (µmol/L)}} = (X^{\text{Jaffe (µmol/L)}} - 8)/1.07$) [16]. To make sure that this conversion would not influence our results, we separated the analyses into the group that donated before 2006 and the group that donated after 2006 and observed no discrepancies between the two groups nor compared with the total cohort (Supplementary data, Table S1). Creatinine clearance was calculated from the 24-h urine collected the day before the measurements.

Kidney function measurements

GFR measurements were performed using ^{125}I-iothalamate and ^{131}I-hippurate infusion as previously described [17]. The day-to-day variability of the mGFR was 2.5% [18]. Measurements were performed in a quiet room, with the participant in semi-supine position. After drawing a blood sample, ^{125}I-Iothalamate and ^{131}I-hippurate infusions was started (0.04 mL/kg containing 0.04 MBq and 0.03 MBq, respectively). At 08:00 h, 0.6 MBq of ^{125}I-Iothalamate was admin-

istered, followed by continuous infusion of 12 mL/h. After a 2-h stabilization period, baseline measurements were performed in a steady state of plasma tracer levels. Clearances were calculated as (*U***V*)/*P* and (*I***V*)/*P*, where *U***V* represents the urinary excretion, *I***V* represents the infusion rate of the tracer and *P* represents the plasma tracer concentration per clearance period. We calculated the mGFR from clearance levels of these tracers using (*U***V*)/*P* and corrected the renal clearance of ^{125}I-iothalamate for urine collection errors by multiplying the urinary ^{125}I-Iothalamate clearances with the ratio of plasma and urinary ^{131}I-hippurate clearance using the following formula:

$$Corrected\ Clearance_{iot} = \frac{Clearance_{hip}(I\ \times V/P)}{Clearance_{hip}(U\ \times V/P)} \times Clearance_{iot}\ (U\ \times V/P)$$

External validation cohort (Mayo Clinic)

For external validation, we used a cohort of 1087 donors from the living kidney donor program of the Mayo Clinic Transplant Center, Rochester, MN, USA. In 1094 donors who donated between 2000 and 2015, the eGFR (using standardized levels of serum creatinine) was obtained prior to donation and mGFR was measured a mean of 6 months after donation. Patients were studied in the fasting (≥4 h) state but were encouraged to keep well hydrated with oral intake of water. An equilibration period of 60 min following the subcutaneous injection of iothalamate contrast was allowed, after which urinary clearances of iothalamate were determined by urine collection over 45–60 min and blood sampling at the beginning and end of that period [15]. Ultrasonographic bladder scanning was performed to ensure complete bladder emptying after all urine collections. Bladder catheterization was performed if residual urine was detected.

Statistical analyses

Data are reported as mean [standard deviation (SD)] for normally distributed variables and median [interquartile range (IQR)] for skewed data. Binary variables are shown as number (%). Post-donation mGFR was indexed for body surface area in all analyses.

Because eGFR is currently most widely used in donor screening for the prediction of post-donation GFR, we first performed descriptive analyses showing which post-donation mGFR values were achieved for the eGFR thresholds of ≥60, ≥70, ≥80, ≥90 and ≥ 100 mL/min/1.73 m^2. The same pre-donation eGFR thresholds were subsequently applied to the external replication (Mayo Clinic) cohort and achieved post-donation mGFR values were calculated and compared

with the TransplantLines cohort findings. We then replicated these analyses in the subgroups of 409 and 110 donors with 5- and 10-year follow-up in the TransplantLines cohort.

Next we used univariable and multivariable linear regression analyses to assess the relationship between pre-donation serum creatinine and post-donation mGFR, adjusting for potential confounders including age, sex and body mass index. Several transformations of pre-donation serum creatinine and/or post-donation mGFR were explored, including square root, inverse, logarithmic, and natural logarithmic transformations, in order to account for potential non-linear associations. For the development of the multivariable model we used a backward approach using the variables with $P < .2$ upon univariable analysis and the variables that were likely to be confounders. This model was subsequently tested in a stepwise forward approach. The final model was used to develop an equation that predicts post-donation mGFR in the development cohort. In order to compare the model to current clinical practice, we developed a basic equation estimating post-donation mGFR as 66% of pre-donation eGFR (defined as the 'reference equation') [12]. The accuracy and precision of both models were assessed in the internal and external validation cohorts. Accuracy was assessed by calculating the mean bias, the model R^2, the root mean square error (RMSE) and the mean percentage bias. Precision was assessed by calculating the IQR of the bias. Furthermore, the percentages of predicted mGFR values within 30% and 10% of the true mGFR value were calculated (P_{30} and P_{10}, respectively). Because mGFR is the gold standard, we also tested the accuracy and precision of the best model including pre-donation mGFR instead of serum creatinine.

Finally, several sensitivity analyses were performed to focus on donors at high risk of a lower mGFR post-donation. First, the accuracy and precision of the optimal model were calculated in a subgroup analysis of donors with a pre-donation eGFR <90 mL/min/1.73 m^2. Second, we identified a subgroup of donors with progressive mGFR loss during longitudinal follow-up. The accuracy and precision of the optimal model were also calculated in this subgroup. Then, since donor age has been increasing over the past years and is likely to continue to increase [19], we assessed the accuracy and precision of the optimal model in a subgroup containing only donors ≥65 years of age. Lastly, we calculated how many donors that reached a post-donation mGFR <50 mL/min/1.73 m^2 or <60 mL/min/1.73 m^2 also had a predicted post-donation mGFR below those thresholds according to equation C and the optimal model. We subsequently calculated the area under the curve (AUC) for model C and the optimal model

to predict a post-donation mGFR <60 mL/min/1.73 m^2. Statistical analyses were performed in SPSS for Windows version 23 (IBM, Armonk, NY), R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism 6 for Windows (GraphPad Software, San Diego, CA, USA). *P*-values <.05 were considered statistically significant.

RESULTS

Characteristics of the development, internal and external validation cohorts

Baseline characteristics of the development and internal validation cohorts (TransplantLines) and external validation cohort (Mayo Clinic) are shown in Table 1. The development cohort consisted of 511 living kidney donors (mean pre-donation eGFR 86 ± 14 mL/min/1.73 m^2). The internal validation cohort consisted of 509 living kidney donors [mean pre-donation eGFR 88 ± 14 mL/min/1.73 m^2 (*P* = .03 versus development cohort)]. The external validation cohort consisted of 1094 living kidney donors [mean pre-donation eGFR 89 ± 15 mL/min/1.73 m^2 (*P* < .001 versus development cohort)]. Baseline characteristics of the subgroups of donors with 5- and 10-year mGFR follow-up available are shown in Supplementary data, Table S2.

Pre-donation eGFR and post-donation mGFR in the development and internal validation cohort

Because eGFR is currently most widely used for the prediction of post-donation GFR, we first illustrated which post-donation mGFR values were achieved for different pre-donation eGFR thresholds in the development + internal validation cohort and in the external validation cohort (Supplementary data, Table S3). A pre-donation eGFR ≥60 mL/min/1.73 m^2, present in 98% of the donors, led to a mean post-donation mGFR of 64 ± 11 mL/min/1.73 m^2 and to an mGFR >48 mL/min/1.73 m^2 in 95% of donors in the development and internal validation cohorts. Donors with a pre-donation eGFR ≥100 mL/min/1.73 m^2 (18%) had a mean post-donation mGFR of 73 ± 9 mL/min/1.73 m^2, but 5% of these donors reached a post-donation mGFR <59 mL/min/1.73 m^2, despite the high pre-donation eGFR. The eGFR thresholds were also tested in the external validation cohort (Supplementary data, Table S3). Here, a pre-donation eGFR ≥100 mL/min/1.73 m^2, present in 24% of the donors, led to an mGFR <56 mL/min/1.73 m^2 in 5% of donors (Supplementary data, Table S3). Thus there is a group of donors that has a pre- to post-donation decrease in GFR of >40%, despite high pre-donation eGFR, which indicates that eGFR alone might not be

sufficient to predict post-donation GFR. Results were similar in a subgroup of 409 TransplantLines donors with 5-year mGFR-based follow-up and a subgroup of 110 TransplantLines donors with 10-year mGFR-based follow-up (Supplementary data, Table S4). We also calculated mean post-donation mGFR values for similar pre-donation mGFR thresholds (Supplementary data, Table S5). Although more donors achieved the pre-donation mGFR thresholds, the mean 3-month post-donation mGFR and the first and fifth percentiles of post-donation mGFR were comparable.

Development of the mGFR prediction model

We subsequently developed several models to predict post-donation mGFR in the development cohort. In univariable analyses, pre-donation mGFR, eGFR (both positive), age, serum creatinine and systolic blood pressure (all negative) were significantly associated with mGFR 3 months after donation (Supplementary data, Table S6). A scatterplot of the association between pre-donation serum creatinine and post-donation mGFR is shown in Supplementary data, Fig. S1, and the association did not change after several transformations (Supplementary data, Table S7). The association between pre-donation serum creatinine and post-donation mGFR remained significant after adjustment for age and sex (Table 2 and Supplementary data, Table S8). Again, transformation of the variables in the models did not improve the linear model (Supplementary data, Table S9). The final model was also tested with pre-donation eGFR/mGFR instead of pre-donation serum creatinine (Supplementary data, Table S10). Similar results were obtained for associations with 5- and 10-year post-donation mGFR outcomes (Supplementary data, Table S11). The unstandardized coefficients from the development cohort were used to develop an equation for the final model predicting the 3-month post-donation mGFR:

$$Post\ donation\ mGFR = 120.13 - 0.33 \times serum\ creatinine - 0.53 \times age - 5.35 \times female$$

The equation for the reference model was as follows:

$$Post\ donation\ mGFR = 0.66 \times pre\ donation\ eGFR$$

Table 1. Characteristics of donors in the development and internal validation cohort (Transplantlines) and external validation cohort (Mayo) before and after donation.

	Development cohort		Internal validation cohort		External validation cohort (Mayo)	
Variable	**Pre-donation**	**Post-donation**	**Pre-donation**	**Post-donation**	**Pre-donation**	**Post-donation**
Number	511	511	509	509	1094	1094
Age, years	53 (11)	53 (11)	53 (11)	53 (11)	45 (12)	46 (12)
Sex, N (% male)	250 (49)		246 (48)		438 (40)	438 (40)
Weight, kg	80 (13)	79 (13)	80 (14)	80 (14)	81 (16)	81 (17)
Length, cm	175 (9)	174 (9)	175 (10)	175 (10)	171 (9)	171 (9)
Body mass index, kg/m^2	26 (3)	26 (3)	26 (4)	26 (4)	28 (5)	28 (5)
Body surface area, m^2	1.95 (0.19)	1.94 (0.19)	1.96 (0.20)	1.95 (0.20)	1.93 (0.22)	1.93 (0.22)
mGFR, mL/min/1.73m^2	101 (15)	64 (11)	101 (17)	64 (11)	102 (18)	66 (13)
$eGFR_{CKD-EPI}$, mL/min/1.73m2	86 (14)	57 (12)	88 (14)	57 (12)	89 (15)	58 (12)
Systolic blood pressure, mmHg	126 (13)	124 (12)	127 (13)	124 (12)	120 (15)	119 (14)
Diastolic blood pressure, mmHg	75 (9)	76 (8)	76 (8)	76 (9)	73 (9)	72 (9)
Proteinuria, g/24 hour	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.00 [0.00-0.00]	0.04 [0.03;0.06]	0.06 [0.04-0.09]
Serum creatinine, µmol/L	79 (14)	114 (21)	77 (14)	114 (22)	79 (14)	112 (34)

Abbreviations: mGFR, measured Glomerular Filtration Rate by iothalamate clearance; eGFR, estimated Glomerular Filtration Rate using the CKD-EPI equation.

Table 2. Multivariable linear regression model of mGFR at three months post-donation in the development cohort.

		St. β	P
Final model (R^2=0.38)	Serum creatinine	-0.42	<0.001
	Age	-0.53	<0.001
	Female sex	-0.25	<0.001

Outcome: three months post-donation mGFR
Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate.

Next, the unstandardized predicted values of the 3-month post-donation mGFR were calculated for both equations. The equation that used pre-donation serum creatinine, age and sex showed good correlation with post-donation mGFR (Fig. 2) and had better accuracy (Table 3, mean bias development cohort 0.41 mL/min/1.73 m^2) and precision (bias IQR development cohort –5.24–5.32 mL/min/1.73 m^2) compared with the reference equation. The model led to less underestimation of post-donation mGFR. We tested for homoscedasticity of the models in Fig. 2 by plotting the unstandardized residuals of model 3 against the unstandardized predicted values and found no correlation between the residuals and predicted values.

Internal and external validation of the living donor prediction models

Performance of the models was subsequently analysed in the internal and external validation cohorts (Table 3). In the internal validation cohort, the new equation showed better accuracy (mean bias –0.51 mL/min/1.73 m^2; indication of an overestimation of post-donation mGFR) and precision (bias IQR –6.00–4.22 mL/min/1.73 m^2), similar to the development cohort. In the external validation cohort, the equations showed higher RMSE, mean bias and bias IQR as compared with the other cohorts, with the new equation showing the lowest RMSE (11.61 mL/min/1.73 m^2), the best precision (bias IQR –4.88–9.14 mL/min/1.73 m^2) and the best accuracy (mean bias 2.56 mL/min/1.73 m^2).

Table 3. Performance of various models for predicting post-donation mGFR in the development, internal validation and external validation cohort.

	Development cohort (n=511)	Internal validation TransplantLines (n=509)	External validation Mayo Clinic (n=1,087)
Reference equation (0.66*eGFR)			
R squared	0.26	0.30	0.20
RMSE	9.80	9.74	14.57
Mean bias	7.05	5.37	7.22
Mean percentage bias	9.87	7.14	8.56
IQR bias	0.19 to 13.31	-1.09 to 11.19	-0.97 to 14.30
P_{30}	93	95	89
P_{10}	43	48	49
New equation (Scr, age, sex)			
R squared	0.38	0.41	0.29
RMSE	8.29	8.45	11.61
Mean bias	0.41	-0.51	2.56
Mean percentage bias	0.97	2.50	1.07
IQR bias	-5.24 to 5.32	-6.00 to 4.22	-4.88 to 9.14
P_{30}	98	96	93
P_{10}	58	60	47

A negative bias indicates an overestimation of post-donation mGFR, a positive bias indicates underestimation.
Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate; RMSE: Root mean square error; IQR: Interquartile range; Scr: serum creatinine.

Sensitivity analyses

We repeated the multivariable regression analyses of pre-donation variables with the 3-month post-donation mGFR in the development cohort using pre-donation eGFR or mGFR instead of serum creatinine (Supplementary data, Table S10). After adjustment for age and sex, similar results were obtained as in the main analyses, although sex was not an independent predictor in these models. Because the model including pre-donation mGFR had a considerably higher R^2 (0.63) than the models containing eGFR (0.37) or serum creatinine (0.38), and mGFR is considered the gold standard, we also developed a prediction model using pre-donation mGFR. This model included pre-donation mGFR and age (sex was not an independent determinant in this model) and the equation of this model is as follows:

$$Post\ donation\ mGFR = 28.83 + 0.46 \times mGFR - 0.22 \times age$$

The accuracy and precision of this equation in the development, internal and external validation cohorts is presented in Supplementary data, Table S12. The mGFR-based prediction model performed better than the serum creatinine–based model in terms of accuracy (mean bias –0.13 mL/min/1.73 m^2 in the internal validation cohort) and precision (bias IQR–4.18–3.34 mL/min/1.73 m^2 in the internal validation cohort). These results were similar in the external validation cohort.

Both equations from the main analyses were tested in a subgroup of donors with a pre-donation eGFR <90 mL/min/1.73 m^2 (Supplementary data, Table S13). In the development cohort, the new equation (serum creatinine, age, sex) had the lowest bias compared with the reference equation (0.35 versus 9.65 mL/min/1.73 m^2, respectively) and bias IQR (–5.21–5.12 mL/min/1.73 m^2 versus 3.18–16.58 mL/min/1.73 m^2, respectively). Similar results were obtained in the internal and external validation cohorts.

In donors with a negative post-donation GFR slope (declining mGFR between 3 months and 5 years post-donation, mean decline –4.79 mL/min, ***n*** = 137), the new equation still had good accuracy (mean bias 4.75 mL/min/1.73 m^2, 5%) and precision (bias IQR 13 mL/min/1.73 m^2) in the internal validation cohort [R^2 was 0.01 lower (0.37)], compared with the main analyses. In a subgroup of donors ≥65 years of age (***n*** = 129), the new equation had a low mean bias of 0.003 mL/min/1.73 m^2 and a bias IQR of 9 mL/min/1.73 m^2 in the internal validation cohort.

Donors with low post-donation mGFR

Of all 1020 donors, 376 reached a 3-month post-donation mGFR <60 mL/min/1.73 m^2. From these donors, 310 (82%) also had a predicted mGFR <60 mL/min/1.73 m^2 according to the reference equation. The new equation predicted an mGFR <60 mL/min/1.73 m^2 in 213 (57%) of these donors. The AUC to predict a post-donation mGFR <60 mL/min/1.73 m^2 was 75% for the reference equation and 82% for the new equation. A 3-month post-donation mGFR <50 mL/min/1.73 m^2 occurred in 85 donors. The reference equation predicted a 3-month post-donation mGFR <50 mL/min/1.73 m^2 in 55 (65%) of these donors, while the new equation only predicted a 3-month post-donation mGFR <50 mL/min/1.73 m^2 in 15 (18%) of these donors.

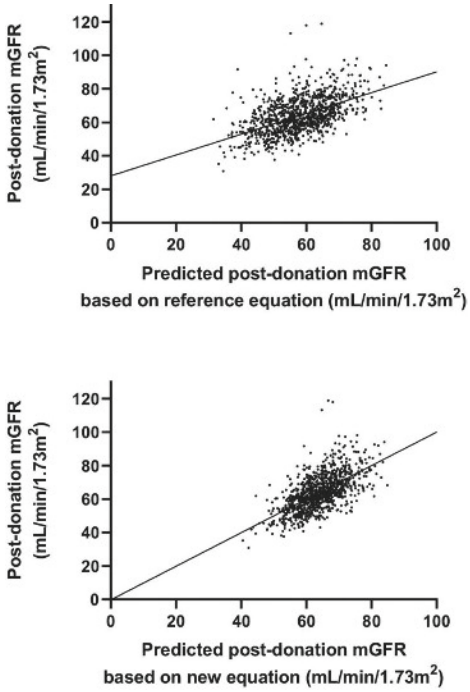

Figure 2. Associations between predicted mGFR and true mGFR by model C (upper figure) and equation 3 (lower figure) in the internal validation cohort.

These differences can be explained by Fig. 2, in which equation 3 clearly has higher accuracy but shows an overall shift to the right, resulting in higher predicted post-donation mGFR values. The 66 donors in which post-donation mGFR was incorrectly classified as >60 mL/min/1.73 m^2 were significantly younger [54 (SD 10) years versus 60 (SD 8) years; $P < .001$] than donors in whom post-donation mGFR was correctly classified as <60 mL/min/1.73 m^2.

DISCUSSION

In this study we show that the prediction of post-donation mGFR improved by using pre-donation serum creatinine, age and sex compared with using only pre-donation eGFR in a Dutch and US cohort of living kidney donors. We developed models that predict short-term post-donation mGFR more accurately and precisely than eGFR alone. The models especially decreased the underestimation of post-donation mGFR. These data can be used to make individualized decisions in donor selection, using pre-donation eGFR without confirmatory testing. Yet, for donors with low pre-donation eGFR, especially young and tall donors, confirmatory testing using a reference method remains necessary.

The need for simple tests that evaluate pre-donation eGFR for the purpose of predicting post-donation mGFR has been highlighted recently [8]. We show that donors with high pre-donation GFR generally have favorable short- and long-term post-donation mGFR. Yet despite the favorable pre-donation GFR, a minority of the donors lose >33% or even >50% of the pre-donation GFR value, indicating that pre-donation GFR alone is not sufficient to predict post-donation mGFR. We show that the accuracy and precision of mGFR prediction can be substantially improved in kidney donors in four models using these additional variables. The models led to less underestimation of post-donation mGFR, which could result in less unjustified rejection of potential donors or less necessity of confirmatory testing. The model developed in this study was validated internally and externally and strongly outperformed the basic model of 66% of pre-donation eGFR (reference model), but showed a somewhat weaker performance in external validation (yet still low bias of <2 mL/min). Also, the new model performed better in donors with a pre-donation eGFR <90 mL/min/1.73 m^2 and in long-term follow-up of donors with progressive kidney function loss after nephrectomy. However, due to systematic overestimation of post-donation mGFR in the lowest range, the model cannot replace confirmatory testing in donors with low pre-donation eGFR, especially in young donors. This is also the case for the reference model, and therefore it is highly important for future studies to investigate which donors benefit from confirmatory testing. A lack of donors with low post-donation mGFR hampers the development of accurate and precise prediction equations in these ranges. These data are in line with other studies proposing the prediction of age-dependent eGFR thresholds in kidney donation [9, 20]. While the model containing pre-donation serum creatinine, age and sex outperformed pre-donation eGFR alone, the model containing pre-donation mGFR and age explained β20% more of the variance in post-donation mGFR than the model based on pre-donation serum creatinine, age and sex. Therefore mGFR still seems the most accurate method for post-donation GFR prediction. When not available, the new model containing pre-donation serum creatinine, age and sex might be a suitable alternative.

To further ascertain long-term renal risks, we repeated our analyses of pre-donation eGFR on long-term post-donation mGFR (5 and 10 years after donation) in a subgroup of donors with available data. Although the numbers were smaller, the prediction model mostly remained similar, indicating that the equations from our models can generally be used for donor screening. It should be noted, however, that while overall the bias of the models was positive (indicating underestimation of 5-year mGFR), the bias was negative for donors with progressive

kidney function loss after donation (indicating overestimation of mGFR). This means that the models might be less suitable to detect donors at risk for kidney function loss after kidney donation, although the overestimation was minor. This is in line with other longitudinal studies on kidney function predictions in kidney patients and living kidney donors: an acceptable performance at the group level, but unable to identify the minority with rapid renal function loss [21] and living kidney donors [22].

Our study can be used in addition to the current donor guideline in donor selection. We propose to individually define a desired post-donation mGFR based on donor risk factors including age, ethnicity and the potential presence of comorbidities [7, 23–27], which could be subsequently translated into an individualized minimum pre-donation eGFR based on the current study. Available donor risk calculators (e.g. www.transplantmodels.com/esrdrisk) can aid with this decision. Donors with an eGFR that will lead to an acceptable post-donation mGFR (e.g. >45 mL/min/1.73 m^2 in a female of 55 years old, based on the lowest reference range for this age and sex [27]) and that do not have contra–indications for donation could be accepted without confirmatory GFR testing. However, caution should be exercised with young or tall donors with low pre-donation eGFR, who might benefit from confirmatory GFR testing. When a very high confidence level and a higher post-donation mGFR are desired, e.g. in younger candidates, only a small percentage of donors have a sufficiently high pre-donation eGFR to be accepted without a confirmatory test. Therefore, measured GFR is still necessary in most of these candidates. Also, in donors at risk for kidney function loss after kidney donation, a confirmatory test is necessary.

A recently published study with pre-donation mGFR measurements in a large number of French donors confirms that the use of eGFR alone may lead to unnecessary rejection of donors [28]. Our study confirms these findings: when a high post-donation mGFR is desired, this comes at the cost of fewer accepted living kidney donors based on eGFR. It should be noted that the current analyses do not take into account long-term adaptive hyperfiltration and the potential development of new-onset kidney disease. Adaptive hyperfiltration can cause GFR to increase up to 10 years after donation and donor chronic kidney disease (CKD) generally develops later after donation and cannot be predicted at a young age [29–32]. We too observe this in an additional analysis in donors with 5- and 10-year mGFR-based follow-up available. At 5- and 10-years after donation, the mGFR remained stable or increased in most donors, indicating that using the short-term post-donation mGFR might be too conservative for living kidney

donor screening. However, in donors with decreasing kidney function, our models showed an overestimation of kidney function. Also, donor comorbidities, CKD risk factors and other factors relevant for the donation-attributable risk should be considered, in particular obesity, as this can elicit maladaptive hyperfiltration and contribute to long-term kidney damage in donors [7, 23–27].

Based on the current state of literature and living donor guidelines, the mGFR is the preferred confirmatory test [26], but it may be expensive and laborious and may vary depending on the method used [35]. Creatinine clearance may be a reasonable alternative: although 24-h urine collection is hampered by sampling errors leading to poor precision, repeated collections may increase its precision and make it a viable confirmatory test [36]. Cystatin-based eGFR has also been proposed as a confirmatory test, but it is considerably less precise in most cases [37, 38]. Further research is necessary to find the best (combination of) tests for confirmation of kidney function if eGFR is below the threshold. By implementing our model of eGFR, age and sex in the selection of living kidney donors, the living donor pool can be extended in centres that do not have mGFR available [26]. Recently the British Transplant Society published a recommendation for (measured) GFR thresholds that can be used when a donor requires confirmatory testing [14].

The strengths of this study include the large number of donors with extensive renal hemodynamic measurements with continuous infusion of ^{125}I-iothalamate and the longitudinal design with 5- and 10-year mGFR-based follow-up in a subgroup of donors. Furthermore, we validated the predictive capacity of pre-donation eGFR in an internal validation cohort and an external validation cohort (Mayo Clinic). Limitations of this study include the absence of non-Caucasian donors in both cohorts and the paucity of data on the very young (age <30 years) and old (>75 years), limiting external validity. Recently a new Chronic Kidney Disease Epidemiology Collaboration equation has been proposed that does not include race [39]. Because of the absence of non-Caucasian donors, we were unable to test this equation in our cohort. Inherent to the donor screening program, older donors were more susceptible to selection bias towards better renal function (and general health), particularly since we only included donors that were accepted for donation. Therefore we are unable to draw conclusions on donors who should be rejected without the use of a confirmatory test.

In conclusion, using two large cohorts of living kidney donors with mGFR before and after donation, we developed a model using a prediction model that includes

pre-donation serum creatinine, age and sex for the prediction of post-donation mGFR during donor screening. This model may be used to identify donors who can be accepted without requiring mGFR confirmation.

ACKNOWLEDGEMENTS

The authors greatly acknowledge all living kidney donors who participated in this study and also appreciate the help of R. Karsten-Barelds, D. Hesseling-Swaving, and M.C. Vroom-Dallinga during the study measurements.

AUTHORS' CONTRIBUTIONS

M.v.L., J.v.d.W., A.D.R. and M.H.d.B. conceived and designed the study. M.v.L., J.v.d.W., S.J.L.B., S.P.B., J.S.F.S., G.N., A.D.R. and M.H.d.B. were responsible for data acquisition. M.v.L., J.v.d.W., R.S.N., A.F.M., S.J.L.B., S.P.B., J.S.F.S., G.N., I.M.N., A.D.R. and M.H.d.B analyzed and interpreted the data. M.v.L., J.v.d.W., R.S.N. and A.F.M. prepared the tables and figures. M.v.L., J.v.d.W., A.D.R. and M.H.d.B. drafted the manuscript and approved the final version. M.v.L., J.v.d.W., R.S.N., A.F.M., S.J.L.B., S.P.B., I.M.N., J.S.F.S., G.N., A.D.R. and M.H.d.B. were responsible for critical revision of the manuscript for important intellectual content and approval of the final version.

FUNDING

M.H.d.B. is supported by a Veni grant from the Dutch Organization for Scientific Research (016.146.014). A.D.R. and A.F.M. were supported with funding from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK090358).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, M.H. De Borst, upon reasonable request.

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SUPPLEMENTARY MATERAL

Table S1. Multivariable linear regression analysis between pre-donation variables and three month post-donation mGFR for the total cohort and separated before and after 2006.

	Total cohort (R^2=0.40)		Donation before 2006 (R^2= 0.35)		Donation after 2006 (R^2=0.43)	
	St. β	P	St. β	P	St. β	P
Serum creatinine	-0.42	<0.001	-0.35	<0.001	-0.51	<0.001
Age	-0.54	<0.001	-0.52	<0.001	-0.51	<0.001
Female sex	-0.25	<0.001	-0.20	<0.001	-0.33	<0.001

Total cohort consists of the development and internal validation cohort.
This table shows that the association between pre-donation eGFR and short-term post-donation mGFR is similar before and after 2006, indicating that the Jaffe conversion of serum creatinine measurements does not affect our results.
Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate; BMI: body mass index.

Table S2. Characteristics of the living donors of the development + internal validation cohort five and ten years after donation.

	Five years	Ten years
Number	409	110
Age, years	57 (10)	61 (9)
Sex, N (% male)	187 (46)	56 (51)
Weight, kg	82 (15)	84 (16)
Length, cm	173 (9)	174 (8)
Body mass index, kg/m2	27 (4)	28 (4)
Body surface area, m2	1.96 (0.20)	1.98 (0.21)
mGFR, mL/min/1.73m2	69 (12)	68 (12)
eGFRCKD-EPI, mL/min/1.73m2	63 (13)	62 (13)
Systolic blood pressure, mmHg	127 (14)	132 (15)
Diastolic blood pressure, mmHg	76 (9)	78 (9)
Proteinuria, g/24 hour	0.06 [0.00-0.60]	0.08 [0.00-0.80]
Serum creatinine, µmol/L	101 (19)	102 (21)

Abbreviations: mGFR, measured Glomerular Filtration Rate by iothalamate clearance; eGFR, estimated Glomerular Filtration Rate using the CKD-EPI equation.

Table S3. Pre-donation eGFR thresholds and three month post-donation mGFR in the development + internal validation cohort and external validation cohort.

	Development + internal validation cohort				
Pre-donation eGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	998	904	713	434	185
Present in % of donors	98%	89%	70%	43%	18%
Post-donation mGFR					
Mean±SD	64±11	65±10	66±10	69±10	73±9
5th percentile	48	49	52	55	59
1st percentile	43	44	48	50	53
	External validation cohort				
Pre-donation eGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	1070	980	776	498	256
Present in % of donors	98%	90%	71%	46%	24%
Post-donation mGFR					
Mean±SD	66±13	67±13	69±13	71±13	75±13
5th percentile	46	47	50	52	56
1st percentile	39	42	44	44	40

Abbreviations: eGFR: estimated glomerular filtration rate, CKD-EPI equation; mGFR: measured glomerular filtration rate.

Table S4. Pre-donation eGFR threshold and five-year post-donation mGFR in the development + internal validation cohort.

	5 year follow-up				
Pre-donation eGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	403	359	266	164	64
Present in % of donors	99%	88%	65%	40%	16%
5 year post-donation mGFR					
Mean±SD	69±12	70±12	71±11	73±11	76±10
5th percentile	51	52	54	57	60
1st percentile	43	47	47	45	58
	10 year follow-up				
Pre-donation eGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	106	85	55	32	7
Present in % of donors	96%	77%	50%	29%	6%
Post-donation mGFR					
Mean±SD	68±12	69±12	72±12	73±12	78±16
5th percentile	51	50	54	51	52
1st percentile	43	43	49	49	52

Abbreviations: eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate.

Table S5. Pre-donation mGFR thresholds and three month post-donation mGFR in the development + internal validation cohort and external validation cohort.

	Development + internal validation cohort				
Pre-donation mGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	1007	996	932	759	496
Present in % of donors	99%	98%	91%	74%	49%
Post-donation mGFR					
Mean±SD	64±11	64±11	65±10	67±10	71±9
5th percentile	48	48	50	54	58
1st percentile	42	44	47	49	54
	External validation cohort				
Pre-donation mGFR	**≥60**	**≥70**	**≥80**	**≥90**	**≥100**
Number of donors	1054	1048	1003	910	735
Present in % of donors	97%	96%	92%	84%	68%
Post-donation mGFR					
Mean±SD	74±18	74±18	75±17	77±17	79±17
5th percentile	49	49	50	53	55
1st percentile	41	41	43	46	49

Abbreviations: mGFR: measured glomerular filtration rate.

Table S6. Univariable linear regression analysis of pre-donation variables on three months post-donation mGFR in the development cohort.

	St. β	P	R^2
mGFR	0.77	<0.001	0.59
eGFR	0.52	<0.001	0.26
Age	-0.51	<0.001	0.26
Serum creatinine	-0.25	<0.001	0.06
SBP	-0.11	0.02	0.01
Sex*	-0.04	0.39	0.00
Weight	-0.04	0.39	0.00
BMI	-0.04	0.32	0.00
Length	-0.01	0.84	0.00
DBP	-0.04	0.92	0.00

*: male=0, female=1
Abbreviations: BMI: body mass index; eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table S7. Association between pre-donation serum creatinine and post-donation mGFR after transformation of one or both of the variables in the development cohort.

	Only serum creatinine transformed		Both serum creatinine and post-donation mGFR transformed	
	St. β	**P**	**St. β**	**P**
Square root transformation	-0.25	<0.001	-0.25	<0.001
Inverse transformation	0.24	<0.001	0.25	<0.001
Logarithmic transformation	-0.25	<0.001	-0.25	<0.001
Natural logarithmic transformation	-0.25	<0.001	-0.25	<0.001

Abbreviations: mGFR: measured glomerular filtration rate.

Table S8. Development of the new prediction model.

		St. β	P	R^2
Model 1	Serum creatinine	-0.25	<0.001	0.06
Model 2	Serum creatinine	-0.29	<0.001	0.37
	Age	-0.56	<0.001	
Model 3	Serum creatinine	-0.42	<0.001	0.38
	Age	-0.53	<0.001	
	Female sex	-0.25	<0.001	

Outcome: post-donation mGFR
Abbreviations: mGFR: measured glomerular filtration rate.

Table S9. Multivariable linear regression analysis of pre-donation variables on log post-donation mGFR in the development cohort.

	St. β	P	R^2
Log serum creatinine	-0.44	<0.001	0.39
Log age	-0.53	<0.001	
Female sex	-0.25	<0.001	

Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate.

Table S10. The new model tested with pre-donation eGFR (model 2) or mGFR (model 3) instead of pre-donation serum creatinine.

		St. β	P	R^2
Model 1	Serum creatinine	-0.42	<0.001	0.38
	Age	-0.53	<0.001	
	Female sex	-0.25	<0.001	
Model 2	eGFR	0.37	<0.001	0.37
	Age	-0.36	<0.001	
	Female sex	0.03	0.41	
Model 3	mGFR	0.68	<0.001	0.63
	Age	-0.22	<0.001	
	Female sex	0.03	0.33	

Outcome: post-donation mGFR
Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate.

Table S11. Multivariable linear regression analysis of pre-donation variables on five-year post-donation mGFR in the development + internal validation cohort.

		Outcome: five year post-donation mGFR			Outcome: ten year post-donation mGFR		
		St. β	P	R^2	St. β	P	R^2
Model 1	Serum creatinine	-0.08	0.13	0.003	-0.13	0.18	0.01
Model 2	Serum creatinine	-0.16	<0.001	0.36	-0.18	0.02	0.36
	Age	-0.60	<0.001		-0.60	<0.001	
Model 3	Serum creatinine	-0.28	<0.001	0.39	-0.28	0.003	0.37
	Age	-0.61	<0.001		-0.60	<0.001	
	Female sex	-0.21	<0.001		-0.17	0.07	

Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate;.

Table S12. Performance of mGFR- or serum creatinine-based models for predicting post-donation mGFR in the development, internal validation and external validation cohort.

	Development cohort (n=511)	**Internal validation TransplantLines** (n=509)	**External validation Mayo Clinic** (n=1,087)
mGFR-based equation (mGFR, age)			
R squared	0.63	0.58	0.49
RMSE	6.39	7.20	12.88
Mean bias	0.16	-0.13	-2.28
Mean percentage bias	-0.71	-1.23	-0.38
IQR bias	-3.75 to 3.42	-4.18 to 3.34	-8.80 to 5.09
P_{30}	99	98	93
P_{10}	73	70	51
New equation (Scr, age, sex)			
R squared	0.38	0.41	0.29
RMSE	8.29	8.45	11.61
Mean bias	0.41	-0.51	2.56
Mean percentage bias	0.97	2.50	1.07
IQR bias	-5.24 to 5.32	-6.00 to 4.22	-4.88 to 9.14
P_{30}	98	96	93
P_{10}	58	60	47

A negative bias indicates an overestimation of post-donation mGFR, a positive bias indicates underestimation.

Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate; RMSE: Root mean square error; IQR: Interquartile range; Scr: serum creatinine.

Table S13. Performance of the living donor models in pre-donation eGFR subgroups in the internal and external validation cohorts for predicting post-donation mGFR from pre-donation data.

	Development cohort		Internal validation cohort		External validation cohort	
	Pre-donation eGFR<90	Pre-donation eGFR≥90	Pre-donation eGFR<90	Pre-donation eGFR≥90	Pre-donation eGFR<90	Pre-donation eGFR≥90
Number of donors	301	210	285	224	589	498
Reference equation (0.66*eGFR)						
R squared	0.12	0.11	0.08	0.16	0.10	0.07
RMSE	9.53	8.96	9.41	9.57	15.49	13.39
Mean bias	9.65	3.28	7.36	2.83	10.22	3.66
Mean percentage bias	14.35	3.45	10.77	2.53	13.98	2.14
IQR bias	3.18 to 16.58	-2.93 to 9.03	0.55 to 12.99	-2.46 to 7.48	1.92 to 16.87	-4.46 to 10.89
P_{30}	89	99	92	98	83	97
P_{10}	33	57	38	61	37	65
New equation (Scr, age, sex)						
R squared	0.33	0.16	0.31	0.21	0.23	0.14
RMSE	8.05	8.63	7.76	9.26	11.02	12.28
Mean bias	0.35	0.51	-0.73	-0.23	2.75	2.34
Mean percentage bias	1.20	0.63	2.94	-1.93	1.69	0.34
IQR bias	-5.21 to 5.12	-5.27 to 5.55	6.31 to 4.27	-5.48 to 3.90	-4.57 to 8.74	-5.39 to 9.70
P_{30}	97	99	97	96	94	93
P_{10}	58	59	56	65	46	48

A negative bias indicates an overestimation of post-donation mGFR, a positive bias indicates underestimation.

Abbreviations: mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate; RMSE: Root mean square error; IQR: Interquartile range; Scr: serum creatinine.

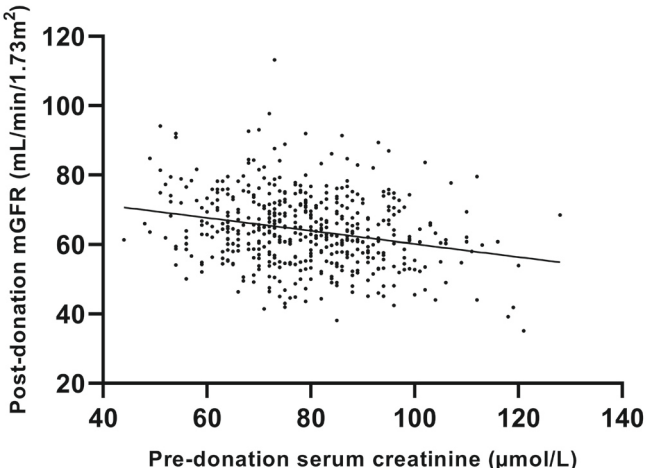

Figure S1. Scatterplot of pre-donation serum creatinine (x-axis) and post-donation mGFR (y-axis) in the development cohort

5

CHAPTER

Microscopic Hematuria at Kidney Donor Screening and Post-Donation Kidney Outcomes

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JOURNAL OF CLINICAL MEDICINE (2022)

ABSTRACT

Although guidelines recommend a kidney biopsy in prospective living kidney donors with unexplained microscopic hematuria, individuals with mild hematuria are commonly allowed to donate without a biopsy. However, the prognostic implications of pre-donation hematuria are unclear. We investigated whether pre-donation microscopic hematuria is associated with changes in post-donation eGFR, proteinuria, or blood pressure. We included 701 living kidney donors with two pre-donation urinalyses and post-donation annual evaluations of the estimated glomerular filtration rate (eGFR), protein/creatinine ratio (PCR), and systolic blood pressure (SBP). The association between pre-donation microscopic hematuria and outcomes was assessed using generalized linear mixed models. The median [interquartile range] follow-up was 5 (2–8) years. Eighty-eight donors had pre-donation microscopic hematuria. There were no significant associations between microscopic hematuria at screening and the course of eGFR (0.44 mL/min/1.73 m^2 increase/year for hematuria donors vs. 0.34 mL/min/1.73 m^2 increase/year for non-hematuria donors (p = 0.65)), PCR (0.02 vs. 0.04 mg/mmol increase/year, p = 0.38), or SBP (1.42 vs. 0.92 mmHg increase/year, p = 0.17) post-donation, even after adjusting for potential confounders. Additional analyses in high-risk subgroups yielded similar results. In this study, pre-donation microscopic hematuria was not associated with post-donation eGFR decline, proteinuria, or hypertension. Microscopic hematuria may reflect primary kidney disease in only a limited subset of donors. Future studies should identify high-risk donor profiles that require further investigation.

INTRODUCTION

Potential living kidney donors undergo extensive evaluation to minimize the risk of post-donation adverse outcomes. Microscopic hematuria is a common finding during donor evaluation since it affects 8–21% of the general population [1,2]. If a urological evaluation is negative, guidelines advise to exclude glomerular causes by kidney biopsy [3,4]. Common glomerular causes of microscopic hematuria include thin basement membrane nephropathy (TBMN), Alport syndrome or a carrier state, and immunoglobulin A (IgA) nephropathy. While TBMN is the most common cause and generally has an excellent prognosis, Alport syndrome and IgA nephropathy are both associated with an increased risk of developing end-stage kidney disease (ESKD) [5,6,7]. Outside the situation of a potential donation, individuals with microscopic hematuria without additional risk factors suggestive of glomerular disease (i.e., proteinuria, increased serum creatinine levels, or hypertension) generally do not undergo kidney biopsy because the renal prognosis is favorable and the biopsy would have no clinical consequences [8,9]. It is not clear whether the prognosis of microscopic hematuria is also favorable in the setting of living kidney donation. There have been some studies on the effect of hematuria on post-donation outcomes, with variable results, and the studies were mostly on a small scale or had limited follow-up [5,10,11,12,13]. Nevertheless, most of these studies agree on the need of a kidney biopsy to exclude glomerular causes before a potential donor can be accepted for donation. In our center, kidney biopsies are not part of the routine living kidney donor evaluation, and therefore in this study we aimed to evaluate whether microscopic hematuria at donor screening is associated with changes in the post-donation course of proteinuria, eGFR, or blood pressure.

METHODS

Study Population

In this prospective cohort study, 701 living kidney donors that donated between 1995 and 2018 in the University Medical Center Groningen were included. We included adult donors who provided informed consent and had undergone at least two urinalyses before and/or shortly after donation. Donors with only dipstick measurements and no erythrocyte counts were excluded. None of the donors underwent kidney biopsy. The clinical parameters of weight, height, and blood pressure and laboratory measures, including serum glucose, were measured at baseline. The studies involving human participants were reviewed and approved by the institutional ethical review board. The participants provided

written informed consent to participate in this study. All procedures were conducted in accordance with the institutional and national ethical standards and the Declaration of Helsinki, as revised in 2013, and the Declaration of Istanbul.

Urinalyses and Definition of Microscopic Hematuria

Microscopic hematuria was defined as ≥1 red blood cell per high-power field (HPF) or ≥3 red blood cells per μL [11]. Microscopic hematuria was judged as present if it was present at least twice within one year before donation or if it was present at least once within one year before donation and once between three months and one year after donation.

Post-Donation Outcomes

After donation, the urinary protein, estimated glomerular filtration rate (eGFR), and systolic blood pressure (SBP) were measured yearly. We used spot urine from freshly voided urine to measure urinary protein and creatinine and calculated the protein/creatinine ratio (PCR) [14]. Serum creatinine was measured by isotope dilution mass spectrometry that was traceable in our biochemical laboratory by enzymatic assay on the Roche Modular (Roche Ltd., Mannheim, Germany) from 1st March 2006. Before this date, samples were measured by the Jaffe alkaline picrate assay on the Merck Mega Analyzer (Merck, Darmstadt, Germany). Values obtained by the Jaffe method were converted to allow comparison with the Roche method by the formula (YRoche = (XJaffe – 8)/1.07) [15]. The CKD-EPI-creatinine formula was used to calculate the estimated glomerular filtration rate (eGFR) [16]. The 15 min automated office measurement was used to determine blood pressure.

Statistical Analyses

Variables with skewed distributions were naturally log-transformed. Because of repeated measurements, we used generalized linear mixed models to investigate the association between pre-donation microscopic hematuria and the changes in post-donation PCR, eGFR, and SBP over time, using individuals as a random effect and an autoregressive covariance structure. We included the interaction term (hematuria×time) to test whether pre-donation hematuria modified the changes in PCR, eGFR, and SBP over time. The models were adjusted for potential pre-donation confounders, including age, sex, blood pressure, body mass index (BMI), eGFR, PCR, and the use of antihypertensive medication.

For the main analyses, we used ≥ 1 red blood cell per high-power field (HPF) or ≥3 red blood cells per μL as the cut-offs for microscopic hematuria based

on a prior study [11], but because other studies used 2–5 red blood cells per high-power field [10,11,12,13,17], we performed sensitivity analyses in which only donors with ≥2 red blood cells per HPF (≥6 per µL) and analyses in which donors with ≥3 per HPF (≥15 per µL) were categorized as "hematuria". We subsequently defined a subgroup of "high-risk" donors and repeated the generalized linear mixed model analyses in this subgroup. Donors were classified as "high risk" if they had at least one of the following risk factors at screening: SBP > 140 mmHG and/or the use of antihypertensive medication, eGFR < age-adapted threshold [18], PCR > 15 mg/mmol, HbA1c > 7%, or BMI > 30. In further sensitivity analyses, we used uni- and multivariable linear regression analyses to investigate the association between pre-donation hematuria and the five-year post-donation eGFR. Lastly, we used latent class growth modeling in an effort to identify a subgroup of patients with a worse progression of the three outcomes over time. A detailed description of the latent class growth analysis is provided below. For each outcome, we coded the group that showed a worse progression over time as "1" and the group that showed a better progression as "0". In uni- and multivariable logistic regression analyses, we investigated whether pre-donation microscopic hematuria predicted a worse progression over time for each outcome. SPSS statistics version 22 (IBM, Armonk, NY, USA) and R version 4.0.4 were used to perform the analyses. p values < 0.05 were considered statistically significant.

RESULTS

Pre-Donation Characteristics of the Living Kidney Donor Population

A total of 177 donors were excluded from donation, of which 9 donors were excluded due to hematuria (**Figure 1**). Details of these donors are shown in **Table S1**. We included 88 (13%) donors with and 613 (87%) donors without hematuria at donor screening (characteristics in **Table 1**). In donors with hematuria, the median [interquartile range] urinary erythrocyte count was 10 [6-22] per µL. In three donors with hematuria, the medical records documented urological analyses, and in all three cases urological causes were excluded. One donor with hematuria had a known history of nephrolithiasis, but no stones were detected at the time of evaluation. In the hematuria group, 38 (43%) donors were relatives of their recipients. The causes of kidney failure in these recipients are shown in **Table S2**. The donor age at donation was 54 (11) in the hematuria group and 52 (11) years in the non-hematuria group (p = 0.18). The hematuria group consisted of more female donors (n = 70 (80%)) than the non-hematuria group (45%, p < 0.001). Of these donors, 44 (63%) were >51 years old. More-

over, donors with hematuria had a higher PCR (9 (0–15) mg/mmol) than donors without hematuria (0 (0–12) mg/mmol, $p = 0.03$). The pre-donation eGFR was similar among the two groups (88 (13) mL/min/1.73 m^2 in the hematuria group vs. 89 (14) mL/min/1.73 m^2 in the non-hematuria group, $p = 0.62$), as was the SBP (125 (11) mmHg in the hematuria group vs. 127 (13) mmHg in the non-hematuria group, $p = 0.30$.). In the hematuria group, 68 out of the 88 donors had microscopic hematuria twice within one year before donation, and 20 had microscopic hematuria once within one year before donation and once between three months and one year after donation. There were no clinically important significant differences in the characteristics between these two subgroups (**Table S3**).

Post-Donation Outcomes

The donor follow-up time was 5 (2–8) years (**Figure 2**). The last available PCR was moderately increased (15–50 mg/mmol) in 121 donors, of whom 15 (12.4%) had pre-donation microscopic hematuria. In 43 donors, the last measured eGFR was <45 mL/min/1.73 m^2, of whom 6 (13.9%) had pre-donation microscopic hematuria. For 195 donors, the last measured SBP was ≥140 mmHg, of which 24 (12.4%) donors had pre-donation microscopic hematuria. The prevalence of microscopic hematuria in these groups was not increased compared to the donors without these outcomes, the total population, or the general population [1,2].

Effect of Hematuria on Long-Term Post-Donation Proteinuria, SBP, and eGFR Course

The mean/median values of post-donation PCR, eGFR, and SBP over time are provided in **Table S4**. The post-donation courses of PCR, eGFR, and SBP were similar among donors with hematuria and those without hematuria (**Figure 1**). Potential differences between the two groups for the three outcomes over time were tested in generalized linear mixed models (**Table 2**). In this table, the upper number (hematuria) represents the difference between the hematuria and non-hematuria group at the first visit after donation (at three months). Time represents the course of the outcome after three months for the non-hematuria group, and time*hematuria represents the difference in the post-donation course of the outcome between the hematuria and non-hematuria group at the first visit after donation (at three months). Time represents the course of the outcome after three months for the non-hematuria group, and time*hematuria represents the difference in the post-donation course of the outcome between the hematuria and non-hematuria group.

Table 1. Baseline characteristics of the living kidney donor population.

		Microscopic Hematuria	
	Total (n = 701)	**Present (n = 88)**	**Absent (n = 613)**
Female sex, n (%)	345 (49)	70 (80)	275 (45) [c]
Caucasian race, n (%)	701 (100)	88 (100)	613 (100)
Living related donations, n (%)	328 (47)	38 (43)	290 (47)
Age, years	52 (11)	54 (11)	52 (11)
Weight, kg	81 (14)	77 (13)	81 (14) [c]
Height, cm	175 (9)	171 (9)	175 (9) [b]
BMI, kg/m^2	26 (4)	26 (3)	26 (4)
BSA, m^2	1.96 (0.20)	1.89 (0.18)	1.96 (0.20) [b]
SBP, mmHg	127 (13)	125 (11)	127 (13)
DBP, mmHg	76 (9)	75 (9)	76 (9)
Hypertension [a], n (%)	183 (26)	23 (26)	160 (26)
Use of antihypertensive medication, n (%)	51 (7)	5 (6)	46 (8)
mGFR, mL/min	115 (22)	111 (22)	115 (22) [b]
$mGFR_{BSA}$, mL/min/1.73 m^2	102 (16)	101 (16)	102 (16)
eGFR, ml/min/1.73 m^2	88 (14)	88 (14)	89 (14)
Serum creatinine, µmol/L	78 (14)	72 (11)	78 (14)[c]
Serum glucose, mmol/L	5.3 (0.6)	5.3 (0.5)	5.3 (0.6)
HbA1C, %	5.5 (0.4)	5.5 (0.3)	5.5 (0.4)
Diabetes, n (%)	6 (1)	1 (1)	5 (1)
Serum cholesterol, mmol/L	5.3 (1.0)	5.3 (1.0)	5.4 (1.0)
LDL	3.5 (0.9)	3.4 (1.1)	3.5 (0.9)
HDL	1.6 (0.5)	1.7 (0.5)	1.5 (0.5)
Triglycerides	1.4 (0.9)	1.2 (0.8)	1.4 (0.9) [b]
Serum urea, mmol/L	5.4 (1.3)	5.3 (1.2)	5.5 (1.3)
Serum potassium, mmol/L	3.9 (0.3)	3.9 (0.3)	3.9 (0.3)
Serum sodium, mmol/L	141 (3)	141 (3)	141 (3) [b]
Sodium excretion, mmol/24 h	195 (73)	172 (66)	199 (73) [b]
PCR, mg/mmol	5 (0–12)	9 (0–15)	0 (0–12)
Erythrocytes per µL	n.a.	10 (6–22)	n.a.

[a]: SBP >140 mmHg and/or DBP >90 mmHg.

[b]: $p < 0.05$ vs. "present" group. c: $p < 0.001$ vs. "pre-sent" group.

Data are presented as means (standard deviations) for normally distributed varia-bles and as medians [first quartile—third quartile] for non-normally distributed variables. Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; PCR: protein/creatinine ra-tio.

Three months after donation, PCR was 0.28 mg/mmol higher in donors with pre-donation hematuria vs. donors without pre-donation hematuria ($p = 0.05$). However, after three months, PCR increased by 0.04 mg/mmol per year in donors with no pre-donation hematuria ($p < 0.001$), while it only increased by 0.02 mg/mmol per year (time + hematuria×time = 0.04 + (–0.02) = 0.02, **Table 2**) in donors with pre-donation hematuria.

There was no significant difference in eGFR three months after donation between the hematuria and the non-hematuria groups (estimate = –1.17, $p = 0.36$). Subsequently, post-donation eGFR increased significantly by 0.34 mL/min/1.73 m^2 per year in donors without pre-donation hematuria ($p < 0.001$, **Table 2**). While post-donation eGFR increased by 0.44 mL/min/1.73 m^2 per year in donors with pre-donation hematuria (time + hematuria×time = 0.34 + 0.10 = 0.44, **Table 2**), the difference in the increase was not significant ($p = 0.65$).

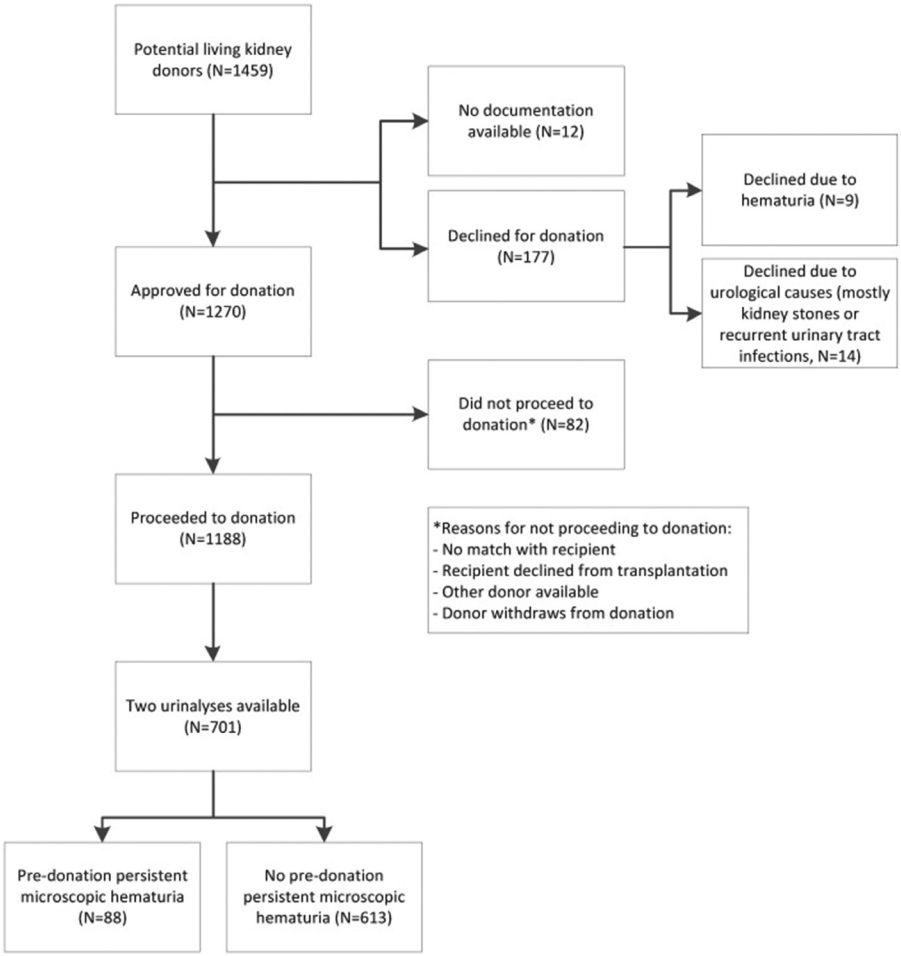
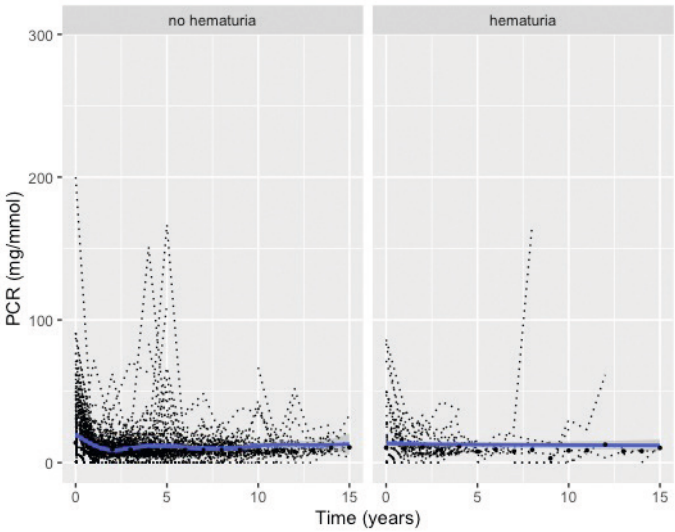
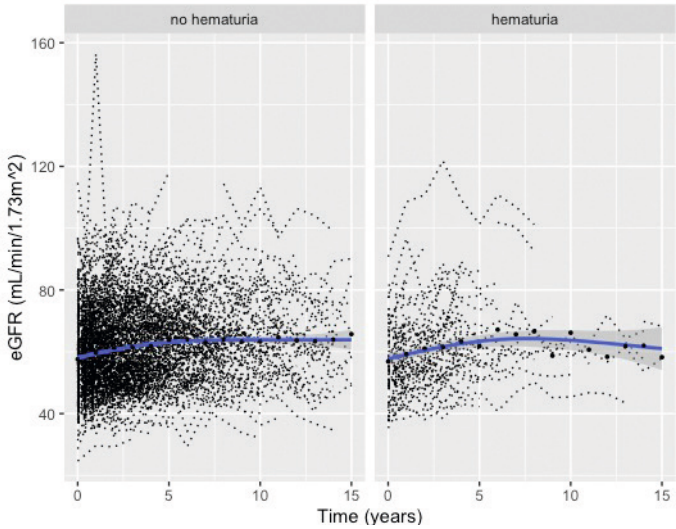


Figure 1. Overview of the study population selection.

Similarly, there was no significant difference in SBP three months after donation (estimate = 1.18, $p = 0.45$). Post-donation SBP increased significantly by 0.92 mmHg per year after donation in donors without pre-donation hematuria ($p < 0.001$, **Table 2**). In donors with pre-donation hematuria, post-donation SBP increased by 1.42 mmHg per year (time + hematuria×time = 0.92 + 0.50 = 1.42, **Table 2**). However, the course of post-donation SBP did not differ significantly between the donors with pre-donation hematuria and those without pre-donation hematuria ($p = 0.17$). The number of donors that used antihypertensive medication at each time point did not materially differ over time (**Table S5**).


no hematuria
hematuria
PCR (mg/mmol)
300
200
100
0
0
5
10
15
Time (years)


no hematuria
hematuria
eGFR (mL/min/1.73m^2)
160
120
80
40
0
5
10
15
Time (years)

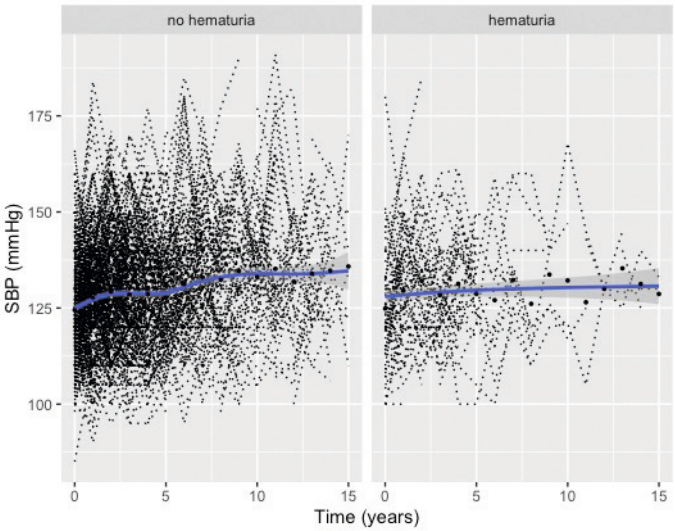


Figure 2. Post-donation course of protein/creatinine ratio (PCR (a)), eGFR (b), and SBP (c) in donors with vs. without pre-donation hematuria.

Smooth curves (blue lines) with 95% confidence intervals (grey areas), individual trajectories (dashed black lines), and mean (median for PCR) values for each time point (black dots).

Table 2. Linear mixed model analysis of the associations between pre-donation hematuria and post-donation PCR, eGFR, and SBP over time.

	Outcome PCR			Outcome eGFR			Outcome SBP		
	Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P
Hematuria [a]	0.28	−0.01 to 0.56	0.05	−1.17	−3.66 to 1.32	0.36	1.18	−1.86 to 4.22	0.45
Time	0.04	0.03 to 0.05	<0.001	0.34	0.23 to 0.44	<0.001	0.92	0.75 to 1.09	<0.001
Hematuria × time	−0.02	−0.08 to 0.03	0.38	0.10	−0.34 to 0.54	0.65	0.50	−0.21 to 1.21	0.17

[a] Donors with pre-donation hematuria were defined as 1, and donors with no pre-donation hematuria were defined as 0. Both models were adjusted for pre-donation age, sex, BMI, eGFR, PCR, SBP, and antihypertensive medication use.
N total = 701. N hematuria group = 88. N non-hematuria group = 613.
Abbreviations: PCR: protein/creatinine ratio; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure.

Table 3. Linear mixed model analysis for the associations between pre-donation hematuria and post-donation ln(PCR), eGFR, and SBP over time in a subgroup of high-risk donors.

	Outcome PCR			Outcome eGFR			Outcome SBP		
	Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P
Hematuria [a]	0.22	−0.19 to 0.64	0.30	−2.64	−6.14 to 0.87	0.14	1.74	−2.45 to 5.92	0.42
Time	0.05	0.03 to 0.06	<0.001	0.24	0.10 to 0.38	0.001	0.80	0.58 to 1.02	<0.001
Hematuria × time	−0.02	−0.10 to 0.06	0.66	0.41	−0.18 to 0.99	0.17	0.002	−0.89 to 0.90	0.996

[a] Donors with hematuria were defied as 1, and donors with no hematuria were defined as 0.
Both models were adjusted for pre-donation age, sex, BMI, eGFR, PCR, SBP, and antihypertensive medication use.
N total = 306. N hematuria group = 41. N non-hematuria group = 265.
Donors were classified as high-risk if one or more of the following CKD risk factors were present: SBP > 140 mmHG and/or the use of antihypertensive medication, eGFR <age-adapted threshold [18], PCR > 15 mg/mmol, HbA1c > 7%, or BMI > 30.
Abbreviations: PCR: protein/creatinine ratio; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure.

Sensitivity Analyses

We performed sensitivity analyses in which only donors with ≥2 red blood cells per high-power field (≥6 per µL, N = 68) or even ≥3 red blood cells per high-power field (≥15 per µL, N = 46) were classified as "hematuria" (**Supplementary Materials**). The results of the generalized linear mixed model analyses with these cut-offs did not reveal increased risks of worse post-donation PCR, eGFR, or SBP courses (**Tables S6** and **S7**). Similarly, the results did not change when analyses were performed in a subgroup of donors with microscopic hematuria twice before donation (**Table S8**).

Generalized linear mixed model analyses were repeated in a subgroup of 306 donors with one or more risk-factors before donation (**Supplementary Materials** and **Table 3**). The baseline characteristics of this subgroup are shown in **Table S9**. Pre-donation hematuria was present in 41 (13.4%) of these high-risk donors. Similar to the total cohort, there was no significant difference in the post-donation course of the outcomes between donors with pre-donation microscopic hematuria and donors without pre-donation hematuria (ln(PCR): difference = −0.02 mg/mmol, $p = 0.66$; eGFR: difference = 0.41 mL/min/1.73 m^2, $p = 0.17$; SBP: difference = 0.002 mmHg, $p = 0.99$).

We performed further sensitivity analyses (**Supplementary Materials**) in a subgroup of 332 donors in whom an eGFR at 5 years post-donation was available. Of this group, 34 (10.2%) donors had pre-donation microscopic hematuria, which was not associated with eGFR at five years after donation (**Table 4**).

Lastly, we defined three subgroups with worse progressions of PCR, eGFR, and SBP over time using a latent class growth analysis (**Supplementary Materials, Results**, and **Figures S2–S4**). Pre-donation hematuria was not associated with a worse post-donation course of PCR or eGFR after adjusting for age, sex, and pre-donation PCR/eGFR (**Table 5**).

Table 4. Uni- and multivariable linear regression analyses of pre-donation hematuria and other characteristics with five-year post-donation eGFR.

	Univariable			Multivariable		
	St. β	**95% CI**	**P**	**St. β**	**95% CI**	**P**
Age, years	−0.52	−0.64 to −0.45	<0.001	-	-	-
Sex, 1 = female	−0.10	−0.21 to 0.01	0.07	0.01	−0.12 to 0.13	0.93
BMI, kg/m^2	−0.01	−0.12 to 0.09	0.80	-	-	-
BSA, m^2	0.07	−0.04 to 0.16	0.22	0.01	−0.11 to 0.12	0.93
eGFR, mL/ min/1.73 m^2	0.59	0.51 to 0.68	<0.001	0.59	0.50 to 0.38	<0.001
SBP, mmHg	−0.08	−0.19 to 0.02	0.13	−0.03	−0.12 to 0.06	0.50
HbA1c, %	−0.08	−0.19 to 0.04	0.19	−0.09	−0.19 to 0.01	0.07
ln(PCR), mg/mmol	−0.07	−0.27 to 0.13	0.49	-	-	-
Hematuria, 1 = positive	−0.05	−0.17 to 0.07	0.40	−0.06	−0.16 to 0.04	0.23

N total = 332. N hematuria = 34. N non-hematuria = 298.
Hematuria and other variables with p < 0.2 in univariable analyses were added to the multivariable model. Abbreviations: eGFR: esti-mated glomerular filtration rate; CI: confidence interval; BMI: body mass index; BSA: body sur-face area; SBP: systolic blood pressure; PCR: protein/creatinine ratio.

Table 5. Uni- and multivariable logistic regression analyses of pre-donation hematuria and worse post-donation outcomes.

	Univariable			Multivariable		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
Outcome PCR group						
Hematuria, 1 = positive	0.71	0.35 to 0.43	0.34	0.49	0.16 to 1.51	0.22
Age	1.00	0.98 to 1.02	0.72	0.99	0.96 to 1.02	0.62
Female sex	0.85	0.56 to 1.31	0.46	0.91	0.42 to 1.97	0.81
Pre-donation PCR	1.03	0.53 to 2.00	0.93	1.11	0.53 to 2.34	0.78
Outcome eGFR group						
Hematuria, 1 = positive	1.23	0.50 to 3.03	0.65	1.45	0.56 to 3.72	0.44
Age	0.98	0.95 to 1.01	0.10	0.96	0.93 to 0.99	0.01
Female sex	0.84	0.44 to 1.59	0.58	0.73	0.37 to 1.45	0.37
Pre-donation eGFR	0.98	0.96 to 1.00	0.11	0.97	0.94 to 0.99	0.01

Table 5. Uni- and multivariable logistic regression analyses of pre-donation hematuria and worse post-donation outcomes. (continued)

	Univariable			Multivariable		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
Outcome SBP group						
Hematuria, 1 = positive	0.65	0.41 to 1.04	0.07	0.63	0.37 to 1.05	0.07
Age	1.00	0.98 to 1.01	0.62	1.02	1.00 to 1.04	0.02
Female sex	0.95	0.70 to 1.28	0.72	0.69	0.49 to 0.97	0.03
Pre-donation SBP	0.94	0.92 to 0.95	<0.001	0.93	0.92 to 0.95	<0.001

Outcome classification was based on a latent class growth analysis in which a group was defined that performed worse than the other group after donation. The group with the poorest outcomes was defined as "1" in the logistic regression analysis, and the group with the best outcomes was defined as "0".
PCR: best post-donation course N = 485, poorer course N = 103.
eGFR: best post-donation course N = 695, poorer course N = 40.
SBP: best post-donation course N = 404, poorer course N = 290.
Abbreviations: CI: confidence interval; PCR: protein/creatinine ratio; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.

DISCUSSION

The present study aimed to investigate whether living kidney donors with pre-donation hematuria were at increased risk of developing post-donation kidney function impairment compared to donors without hematuria. We found no increased risk of developing (progressive) proteinuria in donors with microscopic hematuria at donor screening over a median follow-up time of five years, nor did we find an increased risk of developing an accelerated loss of kidney function or hypertension. Sensitivity analyses in high-risk subgroups showed similar results. These results do not directly support accepting potential donors with hematuria. However, the results pave the way for further studies to identify which donors with hematuria are at increased risk for glomerular disease and would benefit from a kidney biopsy.

The KDIGO guidelines for living kidney donation state that microscopic hematuria requires further evaluation, which may include urinalysis, cystoscopy, a 24 h urine stone panel, or a kidney biopsy. Only donors with a reversible cause may be accepted for donation, and donors with IgA nephropathy should not donate [3]. The British Guidelines for Living Donor Kidney Transplantation state that donors with glomerular disease, detected on kidney biopsy, should not donate, with the possible exception of TBMN [4]. Although individuals with glomerular

disease should not donate, it is unclear in how many patients with microscopic hematuria and no other risk factors for kidney disease on a kidney biopsy will reveal glomerular disease. Outside the setting of living kidney donation, there is an increased long-term risk of ESKD for individuals with microscopic hematuria, but the absolute risk remains very low [17]. The management of these patients is usually not altered by the results of a kidney biopsy, and therefore a kidney biopsy is usually not indicated [8,9]. It is unknown if and/or to what extent unilateral donor nephrectomy changes the risks of microscopic hematuria. In this study, we found no increased renal risk for donors with microscopic hematuria. A kidney biopsy was not performed in the donors with hematuria, which seems to be without consequences in at least the first five years after donation. We would not suggest to never perform a kidney biopsy in potential donors with hematuria. However, we think that these results provide a rationale to discuss and study the position of kidney biopsies in the living kidney donor guidelines.

We observed an initial increase in eGFR over the first five years, followed by a stabilization in the years thereafter, in line with previous studies [19,20]. Our findings may seem to disagree with a previous study by Kido et al. in which pre-donation microscopic hematuria was associated with renal function decline and proteinuria after donation [10]. Differences in the compositions of the cohorts may explain this apparent discrepancy. Kido et al. found that only hematuria with dysmorphic red blood cells was associated with renal function decline and proteinuria. Moreover, in the study by Kido et al., follow-up was only two years, after which renal function was not yet in a steady state, hampering the prediction of long-term risks. In a study by Hassan et al., kidney biopsies were performed in 45 donors with microscopic hematuria [13]. In most donors (*n* = 28), the biopsy results were normal, and in the remaining 17 donors the predominant finding was TBMN (*n* = 13). While the risk of developing ESKD due to TBMN is very low [6], there is no consensus on whether individuals with TBMN can donate [11]. Some studies argue that TBMN is associated with hypertension and proteinuria and that in some cases it could be an expression of the carrier state of Alport syndrome [11]. However, another study showed that living donors with TBMN maintain normal renal function without complications for at least 41 months after donation, and therefore donation with TBMN might be safe [21]. This is different for IgA nephropathy and Alport syndrome, two other relatively common causes of microscopic hematuria [5,7]. The predictors of progression to ESKD for IgA nephropathy are hypertension and proteinuria, but without these conditions the risk of progression of the disease is low [22]. In a study by Nieuwhof et al., biopsy results of 49 patients with microscopic hematuria showed that 12 patients had

IgA nephropathy, 13 had TBMN, 4 had miscellaneous diseases, and the remaining 20 biopsies were normal [23]. More importantly, kidney function remained stable over a median follow-up of 11 years. Studies that investigated biopsies of prospective living kidney donors with microscopic hematuria rarely reported Alport syndrome as a finding, probably because Alport syndrome manifests in an earlier stage in life, is commonly accompanied by extrarenal manifestations, and usually affects other family members as well [7].

It is noteworthy that the majority of donors with hematuria in this study were female, and the suggestion could be made that contamination due to menstruation played a role. However, adjustment for sex did not reveal any significant association between hematuria and any of the outcomes after donation. Furthermore, the majority of the female donors had a post-menopausal age. Another notable difference between the hematuria group and the non-hematuria group was a higher PCR in donors with hematuria. While the values of PCR in the hematuria group were only "moderately increased" [14], this could potentially increase the post-donation risks of kidney function impairment. Despite this finding, we found no increased post-donation risks for donors with pre-donation microscopic hematuria. Nevertheless, these data are too limited and the follow-up was too short to draw conclusions about safety for donors with microscopic hematuria combined with moderately increased PCR, and therefore we would not encourage living donation in such cases without further assessment or a kidney biopsy. The same applies to donors with microscopic hematuria and co-existing hypertension or living related donors with a positive family history for kidney diseases. The assessment of risks of kidney failure or premature death were hampered due to the absence of these events. The results of the current study do not support the acceptance of donors with hematuria without biopsy, which we would therefore not encourage. However, the results suggest that a biopsy might only be advantageous for a subset of donors. Of course, future studies using pre-implantation biopsies and with longer follow-up are warranted to confirm our results. Therefore, future studies should profile donors with hematuria at high risk for glomerular disease and investigate possibilities for alternative testing for glomerular diseases that are less invasive such as genetic testing [24]. This could contribute to identifying potential donors with microscopic hematuria that can be accepted for donation without undergoing kidney biopsy.

The strengths of this study include the relatively large sample size and the extensive post-donation kidney function measurements. On the other hand, the average follow-up duration was limited to five years, and few donors had fol-

low-up data beyond 10 years post-donation. Future studies with more complete long-term follow-up should confirm our results. Moreover, we cannot exclude selection bias since more compliant donors may have more complete long-term data. At the same time, some uncomplicated donors might prefer follow-up by the primary health care provider rather than returning to the transplant center [25]. However, the fraction of available long-term follow-up data was similar between the hematuria and non-hematuria groups. Another source of selection bias was the non-selection of donors with pre-donation hematuria who were declined for donation. In our cohort, nine donors were declined because of (sometimes amongst other reasons) hematuria, and since these donors did not donate, we were not able to assess the risk for these donors. In five of these donors, underlying kidney/glomerular disease was suspected (dysmorphic cells/hypertension/proteinuria/kidney lesion on CT), and in the remaining four there were other comorbidities besides hematuria. Third, the hematuria group was relatively small compared to the non-hematuria group, especially in the sensitivity analyses, and may have been underpowered to detect a small additional risk. On the other hand, we did not find a trend towards worse outcomes in the hematuria group. Moreover, the percentages of donors with microscopic hematuria were consistent in the total population and the high-risk subgroups and matched the prevalence found in the general population [1,2]. Another limitation is that we did not have access to kidney biopsies and more detailed analyses of the urine sediment, and urological and/or other follow-up data were only documented in a few donors. This limitation especially applies to living related donors with hematuria, who may be even more at risk of kidney disease. Lastly, the study only consisted of Caucasian donors, limiting the generalizability to other populations.

In conclusion, we found no differences in the five-year post-donation courses of proteinuria, kidney function decline, or hypertension between carefully selected living kidney donors with microscopic hematuria at donor screening (13% of the population) and living kidney donors without hematuria. These results do not support the acceptance of potential donors with hematuria without performing a kidney biopsy. However, the results provide a rationale to identify which donors with hematuria are at risk and could benefit from a kidney biopsy.

AUTHOR CONTRIBUTIONS

Conceptualization, J.v.d.W., M.v.L., M.H.d.B., and S.P.B.; methodology, J.v.d.W., M.v.L., I.M.N., M.H.d.B., and S.P.B.; software, I.M.N.; formal analysis, J.v.d.W., M.v.L., I.M.N., M.H.d.B., and S.P.B.; investigation, J.v.d.W., and S.P.B.; resourc-

es, J.v.d.W., M.v.L., R.A.P., G.N., J.-S.F.S., M.H.d.B., and S.P.B.; data curation, J.v.d.W., M.v.L., R.A.P., I.M.N., G.N., J.-S.F.S., M.H.d.B., and S.P.B.; writing—original draft preparation, J.v.d.W., M.v.L., R.A.P., I.M.N., G.N., J.-S.F.S., M.H.d.B., and S.P.B.; writing—review and editing, J.v.d.W., M.v.L., R.A.P., G.N., J.-S.F.S., I.M.N., M.H.d.B., and S.P.B.; visualization, M.v.L., I.M.N., M.H.d.B., and S.P.B. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical review and approval were waived for this study by the Institutional Review Board (or Ethics Committee) of the University Medical Center Groningen because the study only included retrospectively collected clinical data from medical records. This is in accordance with national laws. The study was conducted in accordance with the Declaration of Helsinki.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author (S.P.B.). The data are not publicly available due to the privacy of the research participants.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary methods

Latent class growth analysis

SBP, eGFR, and PCR were log-transformed to obtain normally distributed outcome variables. Outliers, defined as values deviating more than four standard deviation from the mean, were removed from the dataset.

We used latent class growth modelling aiming to identify a subgroup of patients with a worse progression over time compared to the rest of them. For this purpose the 'hlme' (heterogeneous linear mixed model) function from the R-package 'lcmm' was used. All models included global and class-specific fixed intercepts and linear and quadratic effects of follow-up time as well as covariates confounders age at donation and gender. The outcome variable were log-transformed eGFR, PCR, and SBP. In addition, four different models were compared: 1) no individual random intercepts, linear or quadratric effects of follow-up time; 2) individual random intercepts, but no linear or quadratric effects of follow-up time; 3) individual random intercepts and linear effects of follow-up time, but no quadratic effects; and 4) individual random intercepts and linear and quadratic effects of follow-up time. Analyses were performed with automated grid searches to run the analyses with ten different starting values to avoid local maxima. For each model the optimal number of classes was determined by the analysis showing the lowest Bayesian Information Criterion (BIC). The best-fitting overall model was regarded the one among the four with the lowest BIC.

Table S1. Description of donors who were declined from donation due to hematuria.

Case number	Findings during donor screening	Red blood cell count	Further evaluation/conclusions
Individuals in whom hematuria was the only reason for exclusion (suspected renal disease)			
1	Glomerular (50% dysmorphic red blood cells) microscopic hematuria on three separate measurements without reduced kidney function, proteinuria or hypertension.	8/µL, 8/µL and 22/µL	Exclusion from donation. No biopsy advised, follow-up hematuria at transplant center.
2	Hypertension, proteinuria (2g/24h) and hematuria (291/µL). Unknown whether dysmorphic erythrocytes were present. Possibly renal disease.	455/µL in spot urine and 291/µL in 24h urine	Exclusion from donation. No biopsy advised, follow-up hematuria at transplant center.

Table S1. Description of donors who were declined from donation due to hematuria. (continued)

Case number	Findings during donor screening	Red blood cell count	Further evaluation/conclusions
3	Glomerular microscopic hematuria (90% dysmorphic red blood cells, no red cell casts).	No count documented	Exclusion from dronation. Follow-up genetic testing revealed a carrier status for Alport syndrome.
4	Glomerular microscopic hematuria (20-40% dysmorphic red blood cells, no cylindric cells). No causes were found at urological evaluation.	24/µL and 153/µL	Exclusion from donation. If continuing the donation procedure is desired, kidney biopsy is needed to exclude glomerular disease.
5	Microscopic hematuria twice during screening without proteinuria, hypertension or reduced kidney function. At urological evaluation, a potentially malignant lesion was seen in the right kidney.	20/µL and 34/µL	Exclusion from donation. Follow-up at urologist for lesion right kidney.
Individuals in whom hematuria contributed to the decision of exclusion amongst other reasons			
6	Low mGFR and erythrocytes in urinesediment. Urine sediment was not repeated during screening due to low mGFR (exclusion from donation anyway).	24/µL	Exclusion from donation. At follow-up by general practitioner, hematuria was no longer present.
7	Possibly SLE and microscopic hematuria on two separate measurements without reduced kidney function, proteinuria or hypertension.	8/µL and 22/µL	Exclusion from donation. Follow-up hematuria at general practitioner was advised.
8	Low mGFR and microscopic hematuria (for which urological evauation had been performed years ago which revealed no urological causes). Besides, a lesion in adrenal glands was found on CT. Lastly unhealthy lifestyle (smoking and alcohol).	4/µL and 5/µL	Exclusion from donation. Follow-up of lesion in adrenal glands was advised.
9	Microscopic hematuria at evaluation (>40% dysmorphic red blood cells). Besides, increased M-protein, alterations on ECG and high blood glucose levels were found.	14/µL and 30/µL	Exclusion from donation. Follow-up of the findings is advised in referral hospital.

Table S2. Causes of kidney failure in recipients who were relatives of donors with pre-donation hematuria.

Cause of kidney failure	N (%)
Focal segmental glomerulosclerosis	4 (11%)
Diabetic nephropathy	4 (11%)
IgA nephropathy	4 (11%)
Vesicoureteral reflux nephropathy	4 (11%)
Anatomical abnormalities limiting urine outflow*	4 (11%)
Assumed consequence of hypertension	4 (11%)
Polycystic kidney disease	3 (8%)
Etiology unknown	2 (5%)
Microscopic polyangiitis	1 (3%)
Alport syndrome (mutation in COL4A5 gene, X-linked)	1 (3%)
Prune belly syndrome	1 (3%)
Kidney atrophy	1 (3%)
Membranous glomerulopathy	1 (3%)
Interstitial nephritis caused by medication use	1 (3%)
Nephrosclerosis caused by familiar hypercholesterolaemia	1 (3%)
Good pasture syndrome	1 (3%)
Granulomatosis with polyangiitis	1 (3%)

*In 1 case caused by Klinefelter syndrome

Table S3. Baseline characteristics of the donors with pre-donation microscopic hematuria.

		Microscopic hematuria	
	Total (n=88)	**Twice before donation (n=68)**	**Once before donation and once after donation (n=20)**
Female sex, n [%]	70 [80]	54 [79]	16 [80]
Caucasian race, n [%]	88 [100]	49 [100]	29 [100]
Age, years	54 (11)	54 (11)	53 (10)
Weight, kg	77 (13)	77 (13)	78 (12)
Height, cm	171 (9)	171 (9)	172 (8)
BMI, kg/m^2	26 (3)	26 (3)	26 (4)
BSA, m^2	1.89 (0.18)	1.89 (0.19)	1.91 (0.16)
SBP, mmHg	125 (11)	126 (11)	122 (12)
DBP, mmHg	75 (9)	75 (9)	73 (8)
Hypertension[∞], n [%]	23 [26]	20 [29]	3 [15]
Use of antihypertensive medication, n [%]	5 [6]	4 [6]	1 [5]

Table S3. Baseline characteristics of the donors with pre-donation microscopic hematuria. (continued)

		Microscopic hematuria	
	Total (n=88)	**Twice before donation (n=68)**	**Once before donation and once after donation (n=20)**
mGFR, ml/min	111 (22)	110 (22)	111 (21)
eGFR, ml/min/1.73m^2	88 (14)	88 (15)	86 (13)
Serum creatinine, µmol/l	72 (11)	72 (12)	73 (9)
Serum glucose, mmol/l	5.3 (0.5)	5.3 (0.5)	5.4 (0.4)
HbA1C, %	5.5 (0.3)	5.5 (0.3)	5.6 (0.3)
Diabetes, n [%]	1 [1]	1 [2]	0 [0]
Serum cholesterol, mmol/l LDL HDL Triglycerides	5.3 (1.0) 3.4 (1.1) 1.7 (0.5) 1.2 (0.8)	5.2 (0.9) 3.3 (1.1) 1.7 (0.5) 1.2 (0.8)	5.6 (1.1) 3.6 (1.5) 1.9 (0.6) 1.3 (0.6)
Serum urea, mmol/l	5.3 (1.2)	5.3 (1.3)	4.9 (1.1)
Serum potassium, mmol/l	3.9 (0.3)	3.9 (0.3)	4.0 (0.3)[a]
Serum sodium, mmol/l	141 (3)	141 (2)	140 (3)
Sodium excretion, mmol/24h	172 (66)	174 (68)	158 (56)
PCR, mg/mmol	9 [0-15]	8 [0-15]	11 [8-14]

[∞]: SBP >140 mmHg and/or DBP >90 mmHg

[a]: P<0.05 vs. "twice before donation" group

Data are presented as mean (standard deviation) for normally distributed variables and as median [first quartile – third quartile] for non-normally distributed variables.

Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate.

Table S4. Long-term follow-up data outcomes.

	Moment after donation															
	3 mo	**1y**	**2y**	**3y**	**4y**	**5y**	**6y**	**7y**	**8y**	**9y**	**10y**	**11y**	**12y**	**13y**	**14y**	**15y**
PCR																
Total population																
Number	363	363	317	279	248	208	144	141	120	94	76	73	39	42	27	10
Median	14	9	9	9	9	8	9	9	9	9	8	9	8	9	9	11
IQR	0-30	6-13	7-13	6-13	6-13	3-14	6-12	7-12	6-13	6-12	6-13	7-12	4-15	7-11	8-17	8-15
Hematuria group																
Number	50	58	45	37	31	18	9	11	8	6	7	6	3	3	4	2
Median	11	10	11	9	11	8	9	8	9	3	8	9	13	8	8	-
IQR	10-28	8-13	6-14	6-13	7-17	4-14	7-13	6-11	2-16	0-14	0-24	7-16	-	-	2-9	-
Non-hematuria group																
Number	313	305	272	242	217	190	135	130	112	88	69	67	36	39	23	8
Median	14	9	9	9	9	8	9	9	9	9	8	8	8	9	10	11
IQR	0-31	6-13	6-13	6-13	6-13	3-14	6-12	7-12	6-12	6-12	6-13	7-12	4-14	7-13	8-17	8-20
eGFR																
Total population																
Number	692	597	488	425	360	332	214	180	140	107	103	83	59	53	30	12
Mean (SD)	58 (12)	60 (13)	61 (13)	62 (13)	62 (13)	64 (13)	64 (14)	63 (13)	64 (14)	63 (13)	64 (15)	64 (13)	63 (14)	63 (14)	64 (14)	64 (14)

Table S4. Long-term follow-up data outcomes. (continued)

	Moment after donation															
	3 mo	**1y**	**2y**	**3y**	**4y**	**5y**	**6y**	**7y**	**8y**	**9y**	**10y**	**11y**	**12y**	**13y**	**14y**	**15y**
Hematuria group																
Number	88	77	60	54	44	34	23	14	11	7	8	6	3	7	4	3
Mean (SD)	57±13	59±13	61±15	62±15	63±14	62±13	67±17	66±18	67±16	59±8	66±18	61±10	-	61±17	-	-
Non-hematuria group																
Number	604	520	428	371	316	298	191	166	129	100	95	77	56	46	26	9
Mean (SD)	58±12	60±13	61±13	62±13	62±13	64±13	64±13	63±12	64±14	63±13	63±14	65±13	64±14	63±14	64±14	66±15
SBP																
Total population																
Number	687	557	458	390	330	321	199	165	134	104	97	82	57	51	30	14
Mean (SD)	124±13	128±14	130±15	129±14	129±14	128±13	131±16	132±17	131±17	135±16	133±15	135±19	132±16	134±14	134±14	132±15
Hematuria group																
Number	86	77	56	51	44	33	19	15	10	7	6	6	3	6	4	3
Mean (SD)	125±14	130±14	132±15	128±13	131±14	129±12	127±18	132±13	126±18	134±10	132±21	127±15	-	135±17	-	-
Non-hematuria group																
Number	601	480	402	339	286	288	180	150	124	97	91	76	54	45	26	11
Mean (SD)	124±13	128±14	129±14	129±14	129±15	128±13	31±16	132±18	132±17	135±16	133±14	135±19	132±16	134±14	135±15	136±17

Table S5. Long-term follow-up data antihypertensive medication (AH med) use.

	Moment after donation														
	1y	**2y**	**3y**	**4y**	**5y**	**6y**	**7y**	**8y**	**9y**	**10y**	**11y**	**12y**	**13y**	**14y**	**15y**
Antihypertensive medication use															
Total population															
N donors	605	496	433	363	336	218	181	143	108	103	84	59	53	30	14
N (%) AH med use	47 (8)	55 (11)	50 (12)	45 (12)	41 (12)	32 (15)	28 (15)	26 (18)	24 (22)	18 (17)	16 (19)	14 (24)	16 (30)	9 (30)	5 (36)
Hematuria group															
N donors	78	61	55	45	34	24	15	11	7	8	6	2	7	4	3
N (%) AH med use	5 (6)	5 (8)	7 (13)	6 (13)	6 (18)	3 (13)	2 (13)	2 (18)	1 (14)	1 (13)	0 (0)	1 (50)	2 (29)	1 (25)	1 (33)
Non hematuria group															
N donors	527	435	378	318	302	194	166	132	101	95	78	56	46	26	11
N (%) AH med use	42 (8)	50 (11)	43 (11)	39 (12)	35 (12)	29 (15)	26 (16)	24 (18)	23 (23)	17 (18)	16 (21)	13 (23)	14 (30)	8 (31)	4 (36)

Table S6. Linear mixed model analysis for the association between pre-donation hematuria (≥2 RBC per high powerfield or ≥6 RBC per µL) and post-donation PCR, eGFR and SBP over time.

	Outcome PCR			Outcome eGFR			Outcome SBP		
	Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P
Hematuria[a]	0.13	-0.16 to 0.41	0.38	-1.28	-3.79 to 1.23	0.32	1.80	-0.93 to 4.52	0.20
Time	0.04	0.03 to 0.05	<0.001	0.34	0.24 to 0.45	<0.001	0.95	0.79 to 1.11	<0.001
Hematuria*time	0.02	-0.06 to 0.10	0.50	0.02	-0.51 to 0.54	0.95	0.67	-0.13 to 1.46	0.10

[a]Donors with pre-donation hematuria were defined as 1, donors with no pre-donation hematuria were defined as 0.
Both models were adjusted for pre-donation age, sex, BMI, eGFR, PCR, SBP and antihypertensive medication use.
N total = 701
N hematuria group = 68
N non-hematuria group = 633
Abbreviations: PCR: protein/creatinine-ratio; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure.

Table S7. Linear mixed model analysis for the association between pre-donation hematuria (≥3 RBC per high powerfield or ≥15 RBC per µL) and post-donation PCR, eGFR and SBP over time.

	Outcome PCR			Outcome eGFR			Outcome SBP		
	Estimate	95% CI	P	Estimate	95% CI	P	Estimate	95% CI	P
Hematuria[a]	-0.04	-0.40 to 0.32	0.84	-1.38	-4.33 to 1.57	0.36	1.17	-2.03 to 4.36	0.47
Time	0.04	0.03 to 0.05	<0.001	0.34	0.24 to 0.44	<0.001	0.96	0.80 to 1.12	<0.001
Hematuria*time	0.08	-0.04 to 0.20	0.19	0.15	-0.54 to 0.84	0.68	0.52	-0.49 to 1.54	0.31

[a]Donors with pre-donation hematuria were defined as 1, donors with no pre-donation hematuria were defined as 0.
Both models were adjusted for pre-donation age, sex, BMI, eGFR, PCR, SBP and antihypertensive medication use.
N total = 701
N hematuria group = 46
N non-hematuria group = 655
Abbreviations: PCR: protein/creatinine-ratio; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure.

Table S8. Linear mixed model analysis for the association between pre-donation hematuria and post-donation PCR, eGFR and SBP over time in a subgroup of donors with hematuria measured twice before donation.

	Outcome PCR			Outcome eGFR			Outcome SBP		
	Estimate	**95% CI**	**P**	**Estimate**	**95% CI**	**P**	**Estimate**	**95% CI**	**P**
Hematuria[a]	0.22	0.11 to 1.73	0.10	-1.26	-3.71 to 1.18	0.31	2.05	-0.58 to 4.68	0.13
Time	0.04	0.03 to 0.05	<0.001	0.33	-0.23 to -0.44	<0.001	0.95	-0.62 to 0.79	<0.001
Hematuria*time	-0.01	-0.07 to 0.04	0.63	0.22	-0.26 to 0.70	0.38	0.09	-0.62 to 0.79	0.81

[a]Donors with pre-donation hematuria were defined as 1, donors with no pre-donation hematuria were defined as 0.
Both models were adjusted for pre-donation age, sex, BMI, eGFR, PCR, SBP and antihypertensive medication use.
N total = 681
N hematuria group = 68
N non-hematuria group = 613
Abbreviations: PCR: protein/creatinine-ratio; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure.

Table S9. Baseline characteristics of the living kidney donor population according to presence of risk factors.

	Total population (n=701)		High risk subgroup* (n=306)	
	Risk factors*		Microscopic hematuria	
	Present (n=306)	Absent (n=395)	Present (n=41)	Absent (n=265)
Female sex, n [%]	152 [50]	193 [49]	34 [83]	118 [44][b]
Caucasian race, n [%]	306 [100]	395 [100]	41 [100]	265 [100]
Age, years	55 (10)	50 (11)b	56 (10)	54 (10)
Weight, kg	85 (15)	77 (12)[b]	79 (14)	85 (15)[a]
Height, cm	175 (10)	176 (9)	169 (9)	175 (10)[b]
BMI, kg/m^2	28 (4)	25 (3)[b]	28 (3)	28 (4)
BSA, m^2	1.99 (0.21)	1.93 (0.18)[b]	1.90 (0.20)	2.01 (0.21)[a]
SBP, mmHg	131 (15)	123 (10)[b]	126 (14)	132 (15)[a]
DBP, mmHg	78 (9)	74 (8)[b]	74 (10)	78 (9)[a]
Hypertension[∞], n [%]	170 [56]	13 [3]	21 [51]	149 [56]
Use of antihypertensive medication, n [%]	51 [17]	0 [0]	5 [12]	46 [17]
mGFR, ml/min	115 (22)	115 (23)	108 (22)	116 (22)[a]
eGFR, ml/min/1.73m^2	87 (13)	90 (14)[a]	86 (13)	87 (14)
Serum creatinine, µmol/l	141 (2)	78 (13)	72 (12)	79 (14)[a]
Serum glucose, mmol/l	5.4 (0.7)	5.2 (0.5)[b]	5.4 (0.6)	5.4 (0.7)
HbA1C, %	5.5 (0.4)	5.5 (0.3)[a]	5.5 (0.3)	5.5 (0.4)
Diabetes, n [%]	6 [2]	0 [0]	1 [2]	5 [2]
Serum cholesterol, mmol/l	5.4 (1.0)	5.3 (1.1)	5.4 (0.9)	5.4 (1.0)
LDL	3.4 (0.9)	3.6 (0.9)	3.0. (1.0)	3.4 (0.9)
HDL	1.6 (0.6)	1.6 (0.4)	1.8 (0.6)	1.5 (0.6)

Table S9. Baseline characteristics of the living kidney donor population according to presence of risk factors. (continued)

	Total population (n=701)		High risk subgroup* (n=306)	
	Risk factors*		Microscopic hematuria	
	Present (n=306)	Absent (n=395)	Present (n=41)	Absent (n=265)
Triglycerides	1.5 (0.9)	1.3. (0.9)[a]	1.3 (0.6)	1.5 (0.9)
Serum urea, mmol/l	5.5 (1.3)	5.4 (1.3)	5.3 (1.2)	5.5 (1.4)
Serum potassium, mmol/l	3.9 (0.3)	3.9 (0.3)	3.9 (0.3)	3.9 (0.3)
Serum sodium, mmol/l	141 (2)	141 (3)	141 (2)	141 (2)
Sodium excretion, mmol/24h	191 (72)	199 (73)	155 (59)	197 (73)[a]
PCR, mg/mmol	10 [0-18]	0 [0-9]	13 [7-19]	9 [0-17]

*: SBP >140 mmHg and/or DBP >90 mmHg

[a]: P<0.05 vs "present" group

[b]: P<0.001 vs "present" group

Data are presented as mean (standard deviation) for normally distributed variables and as median [first quartile – third quartile] for non-normally distributed variables.

Donors were classified as high-risk if one or more of the following CKD risk factors were present: SBP>140mmHG and/or use of antihypertensive medication (n=165), eGFR <age-adapted threshold (18) (n=10), PCR>15 mg/mmol (n=100), HbA1c>7% (n=7) or BMI>30 (n=99).

N no risk factors=395; N one risk factor=237; N 2 risk factors=62; N 3 risk factors=7

Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; PCR: protein/creatinine-ratio.

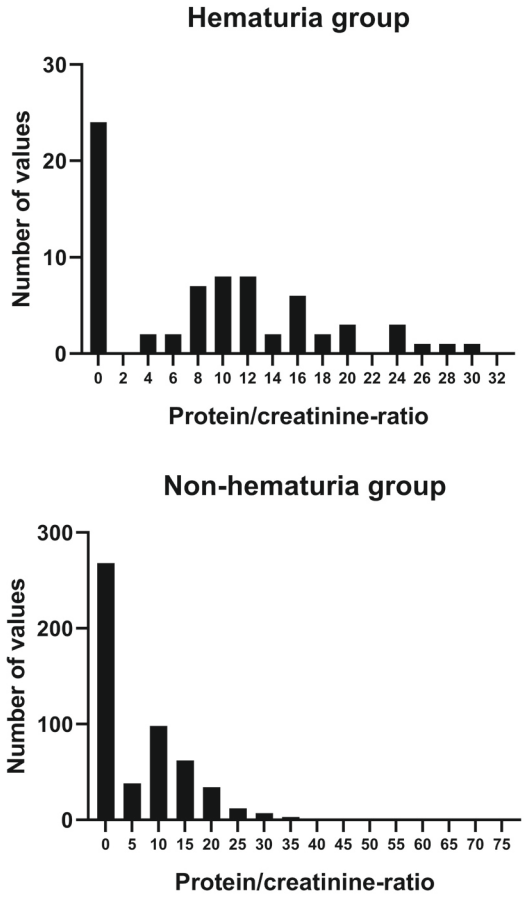

Figure S1. Distribution of pre-donation PCR in the hematuria group and the non-hematuria group.

Upper figure: hematuria group, PCR in mg/mmol. Lower figure: non-hematuria group, PCR in mg/mmol.

SUPPLEMENTARY RESULTS

Latent class growth analysis

For eGFR and SBP, the best fitting model from the latent class growth analyses was the one with two classes and individual random intercepts and linear slopes (Figures x and y). No clear difference is seen in eGFR decline over time between the two eGFR classes. Also for SBP no worse progression is observed for either class. The best fitting model for PCR was the one with four classes and no individual random effects (Figure z). For the 88 individuals in class 1 PCR increases exponentially after five years after donation, while in the other classes it continues to gradually decrease.

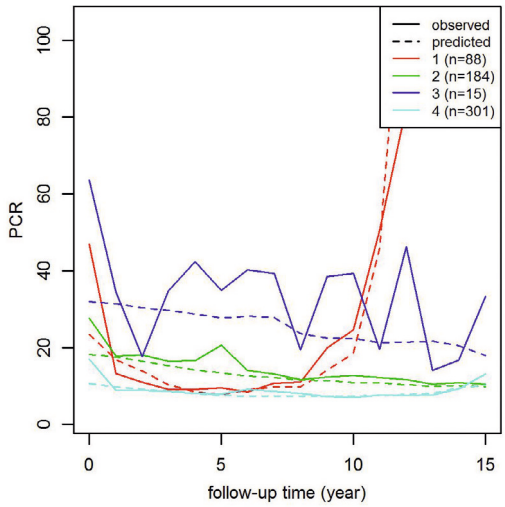

Figure S2. Latent class growth model of post-donation PCR course.

The best fitting model was with four classes of post-donation PCR course. We defined group 1 (red) and 3 (dark blue) as "worse" progressors (=1 in logistic regression analysis) and group 2 (green) and 4 (turquoise) as the group with better post-donation outcomes (=0 in logistic regression analysis).

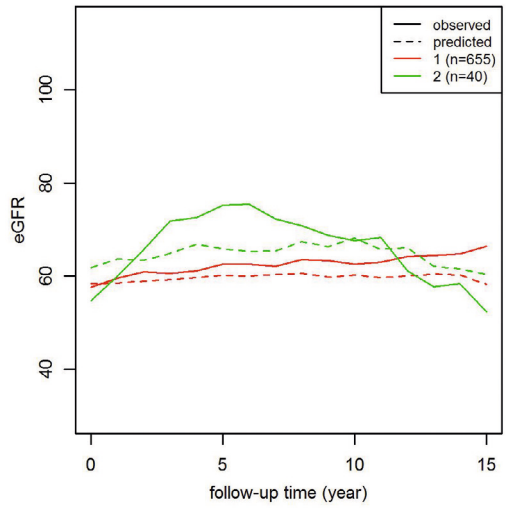

Figure S3. Latent class growth model of post-donation eGFR course.

The best fitting model was with two classes of post-donation eGFR course. We defined group 2 (green) as the "worse" progressors (=1 in logistic regression analysis) compared to group 1 (red, =0 in logistic regression analysis).

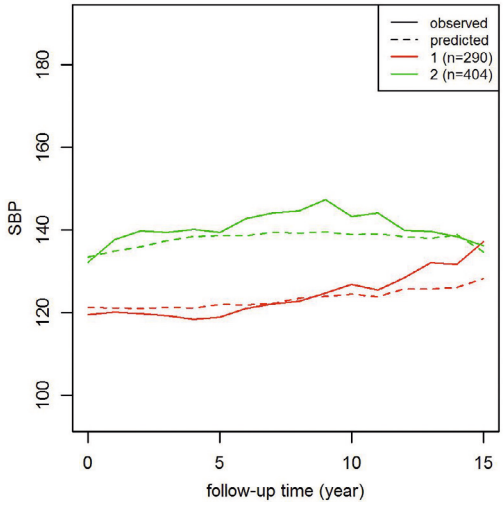

Figure S4. Latent class growth model of post-donation SBP course.

The best fitting model was with two classes of post-donation SBP course. Whereas group 2 (green) had a higher post-donation SBP course, the course remained relatively stable. Group 1 (red) showed an increase over time and therefore we defined this group as "worse" progressors (=1 in logistic regression analysis).

PART

B

6

CHAPTER

Tissue Is the Issue: Kidney Biopsy Findings and Long-term Outcomes in Living Kidney Donors

Jessica van der Weijden, Martin H. de Borst

MAYO CLINIC PROCEEDINGS (2021)

Potential living kidney donors are extensively evaluated to minimize postdonation risks for adverse kidney and cardiovascular outcomes. Although kidney donors in the United States face a 3.5 to 5.3 times higher 15-year observed risk of end-stage kidney disease (ESKD) compared with healthy nondonors, the absolute risk is less than 3% in almost all donors.[1] Yet, optimal predonation assessment of renal risk is essential to improve the efficacy of living donor screening, on the one hand to maintain donor safety and on the other hand to facilitate an adequate donor pool. At the same time, the presence of renal or cardiovascular risk factors at donation can determine the intensity of postdonation follow-up.

Donors with subclinical kidney damage at donation are at risk for development of progressive kidney function loss and eventually ESKD after donation.[2] Clinical parameters including measured glomerular filtration rate (GFR) or estimated GFR (eGFR), age, body mass index, blood pressure, and proteinuria are widely used to identify individuals with established kidney damage or those at high risk for development of future kidney damage. Yet, a substantial part of the variance in postdonation kidney function is not captured by these clinical parameters.[3]

Early kidney damage may remain undetected because of a compensatory increase in GFR in the individual remaining nephrons: the single-nephron GFR.[4] In a study in living kidney donors, both acquired (obesity) and inherent (family history of ESKD) risk factors for chronic kidney disease (CKD) were associated with a higher single-nephron GFR.[5] In addition, a higher single-nephron GFR was associated with larger nephrons and nephrosclerosis on kidney biopsy. Although highly elegant, calculation of the single-nephron GFR requires sophisticated GFR measurements and a stereologically assessed kidney biopsy specimen. Yet, the preimplantation biopsy in itself may already provide important prognostic information because another study from the same group revealed that nephron hypertrophy was associated with higher systolic blood pressure, GFR (possibly reflecting malignant hyperfiltration), and urine albumin excretion.[6] So far, the implications of these microstructural features for long-term kidney outcomes in donors had not been assessed.

In this issue of *Mayo Clinic Proceedings*, Merzkani et al[7] studied a wide range of kidney microstructural features including nephron number, nephron size, and presence of nephrosclerosis from preimplantation biopsy specimens of 807 living kidney donors as potential predictors of postdonation hypertension or CKD. After a mean follow-up of 10.5 years, 6.4% of donors had reached an eGFR below 45 mL/min/1.73 m^2, and 5.1% reported proteinuria. A larger glomerular volume was associated with both outcomes. Interestingly, in a previous study from the same group, larger glo-

merular volume was associated with ***higher*** predonation GFR.[6] These findings can be reconciled by the concept that a relatively high predonation GFR, driven by an increased single-nephron GFR (which was not measured in the current study), may reflect glomerular hyperfiltration in response to an increased demand (eg, obesity) or premature loss of nephrons. Glomerular hyperfiltration, in turn, predisposes to proteinuria and accelerated loss of kidney function.[8] In keeping with this concept, in the current study by Merzkani et al, lower nephron number (below age-specific 5th percentile) was also associated with both an eGFR below 45 mL/min/1.73 m^2 and self-reported proteinuria.

In the context of a pathogenic condition such as diabetes or obesity, glomerular hyperfiltration is considered detrimental. In the setting of living kidney donation, ***adaptive*** hyperfiltration (ie, the compensatory increase in GFR beyond half of the predonation GFR) may not lead to the development of glomerular hypertension and as such may not adversely affect kidney outcomes in donors.[9] Although the underlying mechanisms are not well understood, it may be important to discriminate preexisting glomerular hyperfiltration from postdonation compensatory changes in GFR. A lower nephron number at donation caused by subclinical kidney damage may hamper adaptive hyperfiltration after donation, which could explain the observed association between nephron number and postdonation residual eGFR, defined as the ratio of the postdonation and the predonation eGFR. Yet during donor follow-up, disentangling preexisting malignant hyperfiltration and postdonation adaptive hyperfiltration is highly challenging if not impossible. Here it could be of value to consider microstructural features in the preimplantation biopsy specimen to identify donors with preexisting subclinical kidney injury who are susceptible to development of CKD.

Although the study by Merkanzi and colleagues is sophisticated and based on a unique dataset, the results should be interpreted with caution. The self-reported proteinuria outcome is prone to recall bias, and probably also to selection bias. Furthermore, the use of fixed post-donation GFR thresholds as outcome measures for impaired kidney function in living donors may be questioned. First, there is an ongoing debate on whether CKD thresholds should be adapted to age, given the gradual loss of kidney function with age in the absence of kidney disease.[10] Second, the post-donation GFR should always be considered in the context of donor age, since the impact of a GFR <45 mL/min/1.73m^2 in a 45-year old donor is remarkably different from the same GFR in a 75-year old donor. Third, the implications of a GFR of 45 mL/min/1.73m^2 in a donor are different from those in a CKD patient with the same kidney function. Defining impaired kidney function in living kidney donors will

be challenging due to the low incidence of ESKD post-donation. Analyzing post-donation kidney function as a continuous outcome or repeated GFR measurements could provide additional insight into the predictive value of microstructural features. Finally, some of the results from a prior short-term study[11] could not be reproduced after long-term follow-up. Specifically, the association between arteriosclerosis in the donor biopsy and the development of hypertension at four months after donation was not reproduced after 10.5 years of follow-up. Since it seems unlikely that the impact of arteriosclerosis in the remaining solitary kidney on hypertension risk declines over time, and unfortunately short-term data were not re-analyzed in the current cohort, further studies are warranted to validate these observations.

What are potential implications of this study? The current findings with the limitations addressed here do not justify structural predonation kidney biopsies as part of living donor evaluation. Nevertheless, readily available information from preimplantation biopsies could influence the intensity of donor follow-up in selected cases. Intensified screening for clinically manifest kidney injury (ie, increased albuminuria or loss of kidney function) can trigger early renoprotective interventions to improve donor outcomes. Surrogate markers of preclinical kidney damage might theoretically be feasible for application in clinical practice, but so far no validated markers are available. For example, computed tomography scans can reveal kidney surface roughness, cysts, cortical volume, and cortical to medullary volume ratio that have been associated with nephron number, nephron size, and nephrosclerosis.[6] The potential impact of these markers on postdonation kidney function should be addressed in future studies. The same holds true for markers of nephron number, which has previously been linked with GFR in cross-sectional analyses[6] and is also known to be influenced by birth weight.[12] To the best of our knowledge, there have been no studies linking birth weight, a simple, costless, and noninvasive measure, with postdonation kidney outcomes.

Taken together, the study by Merzkani et al takes a sophisticated approach to identify microstructural features in the preimplantation biopsy specimen that predict long-term kidney outcomes in a large group of living donors. Their observation that low nephron number and larger glomeruli predict a long-term postdonation eGFR below 45 mL/min/1.73 m^2 and self-reported proteinuria contribute to the understanding of how microstructural features of the kidney reflect kidney health and subclinical disease. Moreover, these features might be used to identify donors who need closer follow-up. Future studies should identify circulating, urinary, or imaging markers reflecting subclinical kidney abnormalities to improve donor selection.

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7

CHAPTER

The relationship of peritubular capillary density with glomerular volume and kidney function in living kidney donors

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ACCEPTED FOR PUBLICATION IN JOURNAL OF NEPHROLOGY

ABSTRACT

Background

Peritubular capillary rarefaction plays an important role in the progression of chronic kidney disease. Little is known about the relation between peritubular capillary (PTC) density, glomerular volume and filtration rate (GFR) in the healthy kidney.

Methods

In this single-center study, we included 69 living kidney donors who donated between 2005 and 2008 and had representative renal biopsies available. In all donors, GFR was measured using ^{125}I-Iothalamate before donation and at five years after donation. Before donation, the increase in GFR after dopamine stimulation was measured. Glomerular volume and PTC density were determined in biopsies taken at the time of transplantation. Pearson's correlation coefficient and linear regression were used to assess relations between parameters.

Results

Mean donor age was 52±11 years and mean mGFR was 119±22 mL/min before donation and 82±15 mL/min at five years after donation. While peritubular capillary density (measured by either PTC/50.000μm^2 or PTC/tubule) was not associated with mGFR before or after donation, PTC/tubule was associated with the increase in mGFR after dopamine stimulation (St.β=0.33,p=0.004), and correlated positively with glomerular volume (R=0.24,p=0.047). Glomerular volume was associated with unstimulated mGFR before donation (St.β=0.31,p=0.01) and at five years (St.β=0.30,p=0.01) after donation, independent of age.

Conclusions

In summary, peritubular capillary density was not related to unstimulated renal function before or after kidney donation, in contrast to glomerular volume. However, PTC/tubule correlated with the increase in GFR after dopamine stimulation in healthy kidneys, and with glomerular volume. These findings suggest that PTC density and glomerular volume have different relationships with kidney function in living kidney donors.

INTRODUCTION

Microstructural changes such as glomerular hypertrophy, interstitial fibrosis and tubular atrophy can be present to various degrees in kidneys of healthy individuals without clinical signs of kidney damage [1]. Glomerular volume (GV) is positively associated with single-nephron GFR in healthy individuals, probably as compensation mechanism to maintain a normal total GFR in the case of loss of nephrons or increased renal demand [2]. Moreover, a higher GV is associated with hypertension, overweight, height and a family history of end-stage kidney disease [2,3]. Glomerular enlargement has been explained as the result of either increased intraglomerular pressure or an increased glomerular ultrafiltration coefficient, accompanied by prolongation of glomerular capillaries and subsequent enlargement of the glomerular tuft [4,5]. Indeed, hypertrophic glomeruli have more capillaries, and a greater total capillary area [6,7]. It is unknown whether these glomerular capillary changes also affect the peritubular capillaries (PTC), and if so, whether PTC density is also related to kidney function in the healthy kidney.

The peritubular capillary bed predominantly evolves from the efferent glomerular arteriole [8,9], while the glomerular capillary bed is situated behind the afferent arteriole. A single nephron unit consists of a glomerulus with accompanying tubular system, in which distal tubuli "return" to their own glomerulus, but the PTC microcirculation forms a coalescing plexus surrounding tubuli from different nephrons. Both cortical capillary beds are highly permeable to water and solutes which are filtered in the glomerulus and almost totally reabsorbed via tubuli in peritubular capillaries. They differ in blood pressure as well as in oxygen tension: blood pressure and oxygen levels are high in the glomerulus, while blood pressure is lower and there is a steep decrease in oxygen gradient in the interstitium [9,10]. In patients with insulin-dependent diabetes mellitus, an independent relationship of glomerular and interstitial biopsy parameters with renal function was found [11]. Based on these differences between the glomerular and peritubular capillary beds we hypothesize that an increase in glomerular volume is not accompanied by an increase in peritubular capillaries in healthy kidneys. We expect that in early stages of kidney damage, a phase of glomerular capillary hypertrophy occurs followed by peritubular capillary loss and fibrosis in later stages of chronic kidney disease.

An ideal setting to study microstructural parameters as GV and PTC density in healthy kidneys is in living kidney donors, of whom pre-implantation biopsies are

often available. Previous kidney biopsy studies in living kidney donors showed that glomerular hypertrophy is associated with higher pre-donation GFR [2], but with lower short- and long-term post-donation GFR [12,13]. It also has been shown that a higher body mass index was associated with glomerular hypertrophy [14], and a reduced increase in GFR in response to a dopamine stress test [15]. Thus, in this study, we investigated the relation between PTC density and GV, pre- and post-donation measured GFR in a cohort of living kidney donors.

METHODS

Study population

For this retrospective cohort study, we identified 73 living kidney donors with representative kidney biopsies. Biopsies were taken right after donor nephrectomy (T1), right before implantation (T2) and/or after reperfusion (T3) and were considered representative if T1, T2 and/or T3 had a total cortical surface of minimally 0.6 mm^2 with at least 5 glomeruli. All donors donated between August 11, 2005 and June 17, 2008 at the University Medical Center Groningen, The Netherlands. Four donors were excluded, because they were part of the Dutch "cross-over" program and only came to our center for the actual nephrectomy procedure, rendering 69 living kidney donors eligible for inclusion in this study. All donors underwent pre- and three months post-donation clinical and laboratory measurements as part of the regular living kidney donor screening program. In 52 donors, five-year post-donation follow-up was available. In 2014, these data were added to the TransplantLines Biobank and Cohort study (ClinicalTrials.gov identifier: NCT03272841). This is an observational cohort study on short- and long-term outcomes after organ transplantation/donation, as described previously [16]. The study was approved by the institutional ethical review board (METc 2014/077). All procedures were conducted in accordance with the declaration of Helsinki and declaration of Istanbul.

Biopsy analysis

All available T1, T2 and T3 biopsies were stained with periodic-acid-shiff (PAS) and, on a separate section, an immunohistochemical staining for CD34 (Monosan, Uden, the Netherlands) was performed. In brief, parafin-embedded tissue sections were incubated with primary antibody after blocking of endogeneous peri-oxidase and antigen retrieval by boiling in TRIS EDTA buffer. After washing, the biopsies were incubated with bright vision anti-mouse HRP (Immunologic; Duiven, The Netherlands) followed by washing and thereafter 3,3-diaminobenzidine (DAB) (DAKO cytomation, Glosturp, Denmark) was used as the chromogen.

Thereafter the protocol slides were counterstained with hematoxylin (Klinipath, Duiven, The Netherlands). PAS and immunohistochemically stained slides were digitalized using a Ventana scanner (Ventana iScan HT (Roche, Basel, Switzerland), and imported in Panoramic Image Viewer (3DHistotech, Budapest, Hungary);examples are shown in **Fig. 1**. Microstructural parameters were measured on PAS stained sections by one observer (ML), according to Elsherbiny et al. [14], with the exception that partial glomeruli were counted as 1 and not as 0.5. Briefly, total cortical biopsy area was annotated manually, as well as glomerular tuft surface area of all non-sclerotic glomeruli (NSG). Then the profile area of NSG was calculated by dividing the number of NSG by cortical area. The Weibel Gomez stereological model was used to calculate the NSG density. Furthermore, NSG volume (glomerular volume (GV)) was calculated as described by Elsherbiny et al. [14]. Of all CD34 stained sections a maximum of 10 pictures of 120.000 μm^2 were taken in a serpentine manner [17], with Panoramic Viewer 1.15.4 and exported as jpeg into Paint (Microsoft, Seattle, WA, USA). There were no glomeruli present in these picturesIn all pictures PTCs and tubules were manually traced by one observer (ML), with exclusion of interlobular arteries. Peritubular capillary density was assessed as number of PTCs per tubule (PTC/tubule) and number of PTCs per surface area (PTC/50.000μm^2).The tubular area was determined by dividing the area of the pictures with the number of tubuli counted per biopsy.

In cases that met our inclusion criteria of at least 5 glomeruli and 600.000 μm^2 of cortex, the PAS stained digital section was scored histologically according to Banff by a pathologist (CPK) [18]. Grade of interstitial fibrosis and tubular atrophy (IF/TA) was determined as highest of tubular atrophy (ct) or interstitial fibrosis (ci). Also, IF/TA was assessed by the pathologist as more or less than 5% of the cortical area.

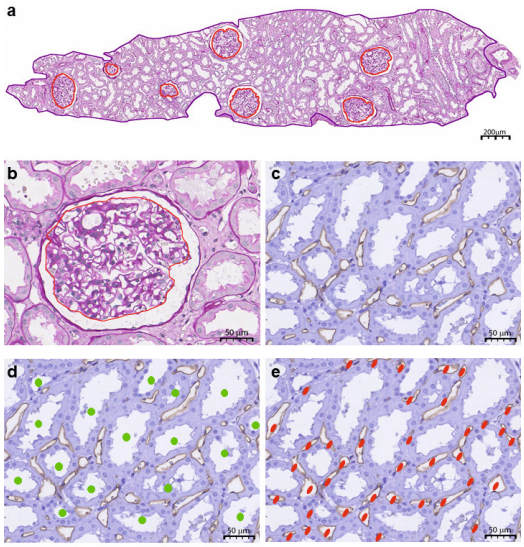


Figure 1. Representative examples of the microstructural measurements on biopsies.

In Periodic acid-Schiff (PAS) stained sections (A and B) the area of cortex was delineated, and the area of the tuft of all individual non-sclerosed glomeruli (depicted in red). On CD34 stained sections (C and D), the peritubular capillaries (PTC) stained in brown, and tubuli (D), were annotated manually.

Assessment of kidney function and other clinical measurements

During screening, clinical parameters as weight, height, hip circumference, waist circumference and blood pressure were measured, medication use was asked as well as smoking history. Kidney function before and at three months and five years after donation was indirectly determined by measuring the clearance of the exogenous filtration marker ^{125}I-iothalamate (measured GFR (mGFR), described in more detail previously) [19]. In short, ^{125}I-Iothalamate and ^{131}I-hippurate infusions were started and after a stabilization period, baseline measurements were performed in a steady state of plasma tracer levels. Clearances were calculated as (U*V)/P and (I*V)/P, where U*V represents the urinary excretion, I*V represents the infusion rate of the tracer and P represents the plasma tracer concentration per clearance period. We calculated mGFR from clearance levels of these tracers using (U*V)/P and corrected the renal clearance of ^{125}I-iothalamate for urine collection errors by multiplying the urinary ^{125}I-Iothalamate clearances with the ratio of plasma and urinary ^{131}I-hippurate clearance by using the following formula:

$$Corrected\ Clearance_{iot} = \frac{Clearance_{hip}(I \times V/P)}{Clearance_{hip}(U \times V/P)} \times Clearance_{iot}\ (U \times V/P)$$

The mGFR after stimulation with dopamine was also assessed before donation ($mGFR_{dopa}$). The $mGFR_{dopa}$ was used to calculate the dopamine-induced increase in GFR ($\Delta mGFR_{dopa}$, previously referred to as the renal functional reserve (RFR) [19,20]) by subtracting the unstimulated mGFR form the $mGFR_{dopa}$. Serum creatinine was measured routinely in our central chemistry laboratory by an isotope dilution mass spectrometry (IDMS) traceable enzymatic assay on the Roche Modular (Roche Ltd., Mannheim, GermanyIn addition serum HbA1c concentration was recorded.

Statistical analyses and sample size estimation

Data are reported as mean (standard deviation (SD)) for normally distributed variables and median [interquartile range, IQR] for skewed data. Binary variables are shown as "number (%)". Correlations between GV, IF/TA, PTC/tubule, tubular area and PTC/50.000µm^2 were assessed by scatter plots and Pearson's correlation coefficients. In cross-sectional analyses, we investigated which pre-donation characteristics were associated with the microstructural parameters using univariable linear regression analyses. Subsequently, we used linear regression analyses to assess the association between the morphometrical parameters and pre- and post-donation kidney function outcomes. Outcomes were pre- and three months and five year post-donation mGFR. All univariable associations of the microstructural parameters with pre- and post-donation outcomes were adjusted for age using multivariable linear regression analyses, because age is a known determinant of GFR as well as mucrostructural features in the kidney [2,21]. To detect a correlation of 0.3 with an β of 0.05 and a power of 80%, 67 donors are needed. Statistical analyses were performed in SPSS version 28 for Windows (IBM, Armonk, NY), and Graphpad Prism 8 for Windows (Graphpad, San Diego, CA). P-values of <0.05 were considered statistically significant.

RESULTS

Pre- and post-donation characteristics

A total of 69 living kidney donors were included in this study. Mean age was 52±11 years, 46% was female and all donors were white (**Table 1**). The donors had a mean body mass index (BMI) of 26±4 kg/m^2 and a mean systolic blood pressure (SBP) of 130±15 mmHg. Three donors had a pre-donation serum HbA1c level ≥6.5%, of which two donors had a BMI of 34 and 35 kg/m^2 respectively. Pre-donation mGFR was 119±22 mL/min and decreased to 75±14 at three months post-donation (Table S1). Five years after donation, mGFR was 82±15 mL/min. Before donation, mean GV was 0.0024±0.0007 mm^3, mean number of

PTC/tub was 1.97±0.3, mean number of PTC/50.000 μm^2 was 25.9±4.4, mean tubular area was 3679.2±835.7 μm^2, and 19 donors had >5% IF/TA (**Table 2**).

Table 1. Baseline characteristics of the living kidney donor population

N	69
Age, years	52±11
Sex, N (%} female	33 (46)
Race, N (%) white	69 (100)
Weight, kg	81±13
Length, cm	176±8
BMI, kg/m^2	26±4
BSA, m^2	1.97±0.17
Hip size, cm	97±7
Waist size, cm	92±9
Waist/hip-ratio	0.95±0.08
SBP, mmHg	130±15
DBP, mmHg	77±9
Serum HbA1c, %	5.7±0.8
Serum creatinine, mmol/L	79±13
Smoking, N (%) smokers	23 (32)

Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Microstructural characteristics of the donor kidneys

N	69
Number of non-sclerotic glomeruli (n)	17.1 ± 8.6
Cortical area (mm2)	6.0 ± 2.6
NSG area, *(μm2)*	20879±10266
Glomerular volume (mm3)	0.0024 ± 0.0007
Glom area density (glomeruli/mm2)	2.92 ± 0.92
Glomerular density (glomeruli/mm3)	19,68 ± 7,49
Profile tubular area, um2	3679.2 ± 835.7
PTC/tubule	1.97 ± 0.3
PTC/50.000μm^2	25.9±4.4
Any tubular atrophy	58 (73%)
IF/TA >5%	19 (24%)

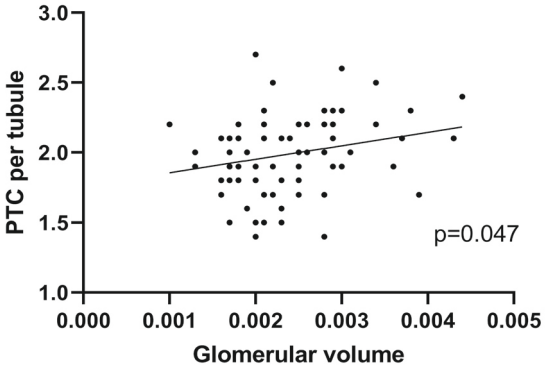
Abbreviations: IF/TA: interstitial fibrosis/tubular atrophy; NSG: non-sclerotic glomeruli; PTC: peritubular capillary.

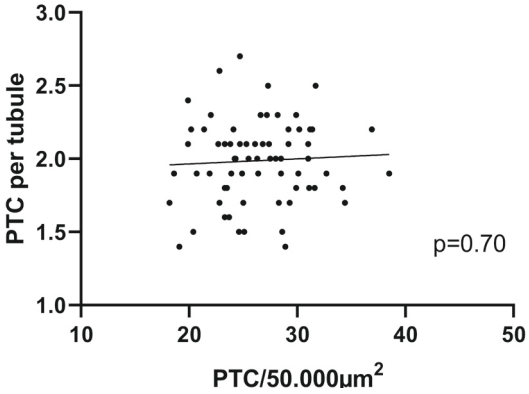
Correlations between microstructural parameters

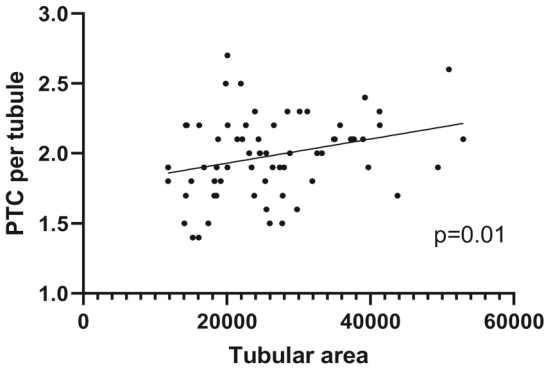
Scatterplots of correlations between microstructural parameters are shown in **Fig. 2**. The strongest correlation was observed for tubular area with PTC/50.000 μm^2 (R=-0.63, P<0.001), with less PTCs per 50.000 μm^2 in cases with larger tubular area. However, when the number of PTCs was adjusted for the number of tubules on the biopsy (PTC/tubule), we observed an increase in PTC/tubule in cases with increased tubular area (R=0.31, p=0.01), which is as expected because cases with larger tubules display a smaller number of tubules per surface area on the biopsy. Glomerular volume correlated positively with tubular area (R=0.26, p=0.03) and with PTC/tub (R=0.24, p=0.047), and negatively with a trend towards significance with PTC/50.000 μm^2 (R=-0.21, p=0.08). There was no correlation between PTC/tubule and PTC/50.000 μm^2 (R=0.05, p=0.70).

Clinical determinants of microstructural parameters in living donor kidney biopsies

Univariable linear regression analyses did not reveal associations of clinical variables (e.g. age, sex, weight, blood pressure) with PTC/tubule or PTC/50.000μm^2 (**Table 3**). Body surface area (BSA) (St.β=0.30, p=0.01), waist/hip-ratio (St.β=0.25, p=0.05), systolic blood pressure (SBP, St.β=0.35, p=0.004) and diastolic blood pressure (DBP, St.β=0.30, p=0.01) were all positively associated with GV (**Table 3**). A trend towards significance was shown for the association of BMI with GV (St.β=0.23, p=0.06). Smoking correlated negatively and significantly with tubular area (St.β=-0.38, p=0.004). Living kidney donors with IF/TA>5% in their biopsy were older than donors without IF/TA (t-test p=0.002, **Table S2**). Also, individuals with IF/TA>5% had a larger tubular area (Table S2). None of the clinical parameters were associated with PTC/tubule or PTC/50.000μm^2.


PTC per tubule
3.0
2.5
2.0
1.5
1.0
0.000
0.001
0.002
0.003
0.004
0.005
Glomerular volume
p=0.047


PTC per tubule
3.0
2.5
2.0
1.5
1.0
10
20
30
40
50
PTC/50.000µm2
p=0.70


PTC per tubule
3.0
2.5
2.0
1.5
1.0
0
20000
40000
60000
Tubular area
p=0.01

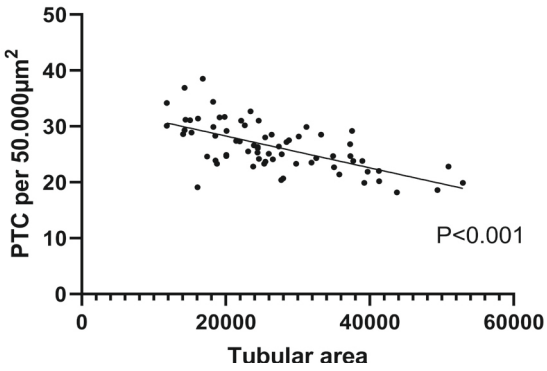


Figure 2. Scatter plots of the morphometrical parameters with eachother.

Upper left: glomerular volume (x-axis) with PTC/tubule (y-axis), Pearson=0.24, p=0.047;
Upper right: glomerular volume (x-axis) with PTC/50.000µm² (y-axis), Pearson=-0.21, p=0.08;
Middle left: glomerular volume (x-axis) with tubular area (y-axis), Pearson=0.26, p=0.03;
Middle right: PTC/50.000µm² (y-axis) with PTC/tubule (x-axis), Pearson=0.05, p=0.70;
Lower left: tubular area (y-axis) with PTC/tubule (x-axis), Pearson=0.31, p=0.01;
Lower right: tubular area (y-axis) with PTC/50.000µm² (x-axis), Pearson=0.63, P<0.001.

Table 3. Univariable linear regression analysis of pre-donation variables with morphometrical parameters.

	PTC/tubule		PTC/50.000µm²		Glomerular volume		Tubular area	
	St.β	p	St.β	p	St.β	p	St.β	p
Age	0.09	0.47	-0.15	0.23	0.04	0.78	0.18	0.16
Sex	-0.17	0.15	0.05	0.67	-0.14	0.25	-0.13	0.29
BMI	0.06	0.64	-0.03	0.83	0.23	0.06	-0.09	0.46
BSA	0.18	0.13	-0.09	0.48	0.30	0.01	0.10	0.43
Waist/hip-ratio	0.17	0.18	-0.02	0.88	0.25	0.05	-0.11	0.42
SBP	0.17	0.17	-0.17	0.19	0.35	0.004	0.21	0.09
DBP	0.01	0.99	-0.17	0.17	0.30	0.01	0.10	0.43
Serum HbA1c	0.03	0.85	0.25	0.06	0.13	0.33	-0.13	0.34
Serum creatinine	-0.04	0.72	0.06	0.62	-0.07	0.55	-0.06	0.63
Smoking	-0.17	0.21	0.20	0.14	-0.09	0.50	-0.38	0.004

Abbreviations: BMI: body mass index; BSA: body surface area; DBP: diastolic blood pressure; PTC/tubule: peritubular capillary per tubule; PTC/50.000µm²: peritubular capillary per 50.000µm²; SBP: systolic blood pressure.

Associations of microstructural parameters with pre-donation GFR

PTC/tubule was significantly and independent of age associated with the $\Delta mGFR_{dopa}$ (=dopamine induced increase in mGFR, St.β=0.25, p=0.04, **Table 4**), but not with unstimulated mGFR (St.β=0.17, p=0.14). PTC/50.000μm^2 was not associated with mGFR or $\Delta mGFR_{dopa}$ (St.β=0.01, p=0.97 and St.β=0.04, p=0.74, respectively). Glomerular volume was significantly and positively associated with pre-donation mGFR (St.β=0.31, p=0.01, **Table 4**), but not with the $\Delta mGFR_{dopa}$ (St.β=-0.13, p=0.31). Tubular area and IF/TA were not associated with pre-donation kidney function (**Table 4**). In a multivariable linear regression model including GV and PTC/tubule, both were independently associated with pre-donation $mGFR_{dopa}$ (R^2=0.29), with GV being associated with pre-donation mGFR, and PTC/tub with pre-donation $\Delta mGFR_{dopa}$ (**Table S3**). The association of PTC/tubule with $mGFR_{dopa}$ and $\Delta mGFR_{dopa}$ remained significant after adjustment for tubular area (PTC/tubule with $mGFR_{dopa}$: St.β=0.29, p=0.01; PTC/tubule with $\Delta mGFR_{dopa}$: St.β=0.26, p=0.045, **Table S4**).

Associations of microstructural parameters with post-donation GFR

There was no association of PTC/tubule with unstimulated mGFR at three months or five years post-donation. Glomerular volume was significantly and positively associated with both three months, and five years post-donation mGFR (St.β=0.27, p=0.02 and St.β=0.30, p=0.01 respectively, **Table 5**). Tubular area, PTC/50.000μm^2 and IF/TA were not associated with post-donation mGFR (**Table 5**).

Table 4. Association of microstructural parameters with pre-donation kidney function.

Independent variable	Outcome	St.β	p	R^2
PTC/tubule	Pre-donation mGFR	0.17	0.14	0.15
	Pre-donation $mGFR_{dopa}$	0.33	0.004	0.23
	Pre-donation ΔmGFRdopa	0.25	0.04	0.06
PTC/50.000μm^2	Pre-donation mGFR	0.01	0.97	0.12
	Predonation $mGFR_{dopa}$	0.00	0.99	0.13
	Pre-donation ΔmGFRdopa	0.04	0.74	-0.004
Glomerular volume	Pre-donation mGFR	0.31	0.01	0.22
	Pre-donation $mGFR_{dopa}$	0.30	0.01	0.22
	Pre-donation ΔmGFRdopa	-0.13	0.31	0.01
Tubular area	Pre-donation mGFR	0.14	0.24	0.14
	Pre-donation $mGFR_{dopa}$	0.21	0.08	0.17
	Pre-donation ΔmGFRdopa	0.04	0.79	-0.01

Table 4. Association of microstructural parameters with pre-donation kidney function. (continued)

Independent variable	Outcome	St.β	p	R^2
IF/TA	Pre-donation mGFR	-0.11	0.35	0.13
	Predonation $mGFR_{dopa}$	-0.10	0.43	0.13
	Pre-donation ΔmGFRdopa	-0.17	0.19	0.02

All analyses adjusted for age, $\Delta mGFR_{dopa} = GFR_{dopa}$ – GFR (=dopamine induced GFR increase, in literature referred to as "renal functional reserve"). Abbreviations: mGFR: measured glomerular filtration rate; $mGFR_{dopa}$: measured glomerular filtration rate after stimulation with dopamine; PTC/tubule: peritubular capillary per tubule; PTC/50.000µm^2: peritubular capillary per 50.000µm^2; IF/TA: interstitial fibrosis and tubular atrophy.

Table 5. Association of microstructural parameters with post-donation kidney function.

Microstructural parameter	Outcome	St.β	p	R^2
PTC/tubule	3 months post-donation mGFR	0.14	0.22	0.15
	5 years post-donation mGFR	0.18	0.10	0.39
PTC/50.000µm^2	3 months post-donation mGFR	0.06	0.64	0.13
	5 years post-donation mGFR	-0.05	0.67	0.35
Glomerular volume	3 months post-donation mGFR	**0.27**	**0.02**	0.20
	5 years post-donation mGFR	**0.30**	**0.01**	0.44
Tubular area	3 months post-donation mGFR	0.14	0.25	0.15
	5 years post-donation mGFR	0.08	0.50	0.36
IF/TA	3 months post-donation mGFR	-0.14	0.26	0.14
	5 years post-donation mGFR	0.05	0.64	0.35

All analyses adjusted for age.
5 years post-donation mGFR available: N=52
Abbreviations: mGFR: measured glomerular filtration rate; mGFRdopa: measured glomerular filtration rate after stimulation with dopamine; PTC/tubule: peritubular capillary per tubule; PTC/50.000µm2: peritubular capillary per 50.000µm2; IF/TA: interstitial fibrosis and tubular atrophy.

DISCUSSION

The present study aimed to investigate the relationship between peritubular capillaries density and other microstructural parameters including glomerular volume (GV), tubular area and IF/TA in healthy kidneys. Furthermore, we investigated whether PTC density and other microstructural parameters were associated with clinical characteristics and pre- and post-donation measured GFR. In this study we confirm associations of GV with mGFR, systolic blood pressure and body size measurements at donation. We found no association of PTC density (measured by either PTC/50.000eenheid or PTC/tubule) with clinical characteristics or pre- or post-donation mGFR. However, we did find a positive association between PTC/tubule and $\Delta mGFR_{dopa}$. Our results indicate that glomerular volume and peritubular capillary density have a differential relationship with kidney function. In addition, our findings suggest that an increase in glomerular capillaries (i.e. glomerular volume) is not associated with an increase in number of peritubular capillaries in healthy individuals. Peritubular capillary density may therefore not provide prognostic information in potential living kidney donors.

It has been broadly recognized that peritubular capillary rarefaction plays an important role in the development of interstitial fibrosis and tubular atrophy (IF/TA) and the progression of chronic kidney disease (CKD) [22–25]. In recipients of a kidney from a deceased donor, an average decrease in the PTC/tubule ratio of nearly 25% the first three months after transplantation is associated with lower graft function [7]. Gaining knowledge on how PTCs react to early compensatory/pathological microstructural changes in the kidney can contribute to better understanding their role in the development of CKD. We observed a negative correlation (with trend towards significance) between PTC/50.000μm^2 and GV, i.e. larger glomerular volume is associated with less peritubular capillaries in the pre-implantation biopsy. In a case report of two cases with low birth weight (known to be associated with low nephron number and CKD), proteinuria and polycythemia, a decreased PTC per surface area was also found together with glomerular hypertrophy [26].The association between GV and tubular area that we observed was in line with previous findings [14]. The positive relationship of GV with PTC/tubule that we found is likely due to a combination of a decrease in PTC density and an increase in tubular area (i.e. less tubules per picture) in individuals with larger glomeruli. Experimental studies show that even subtle alterations in tubular cells [27] or pericytes [28,29] can induce PTC loss and IF/TA, indicating that the tubulovascular ratio (measured by PTC/tubule) provides additional information next to counting PTC numbers per surface area.

Even though PTC density is clearly decreased in advanced CKD [22–25], we found no association of PTC density with kidney function in our cohort, possibly because only healthy kidneys with normal GFR were included in this study. Total GFR is the result of single nephron GFR and number of nephrons [2], so it would be interesting for future studies to investigate whether PTC density is in fact related to single-nephron GFR, and whether this explains the lack of an association with total GFR in healthy kidneys. Our finding that IF/TA in the pre-donation biopsy is not related to mGFR post-donation confirms results from Buus et al. [30]. We observed that individuals with more than 5% IF/TA had an increased tubular area and (a trend towards) a larger glomerular volume. In biopsies of patients with IgA nephropathy and various forms of chronic tubulointerstitial disease, hypertrophic tubuli expressed vascular endothelial growth factor (VEGF), which did not protect from PTC loss with concomitant loss of renal function [31,32]. Further studies are needed to investigate whether tubular hypertrophy may be a first response to glomerular enlargement in healthy individuals, that, if not compensated for by an increase in PTC density, might lead to decreased tubular oxygen supply resulting in tubular atrophy, PTC loss, interstitial fibrosis, and renal function decline.

While PTC density was not associated with pre- or post-donation mGFR, we did find an association between PTC/tubule and the GFR increase after dopamine infusion ($\Delta mGFR_{dopa}$). Dopamine infusion induces dilatation of the afferent and efferent arterioles, and the GFR increase after dopamine infusion has been referred to as “renal stress testing” [19]. As hypothesized by Van Londen et al., the $\Delta mGFR_{dopa}$ may be a measure of the hemodynamic response range of the kidney [19]. It could be that loss of PTC/tubule goes hand-in-hand with an overall decreased tubulovascular health in the kidney, resulting in a diminished hemodynamic response to dopamine infusion, but more detailed data on renal hemodynamics are needed to further substantiate this. This would be in line with the hypothesis from R. Johnson et al. that subtle tubulointerstitial injury with PTC rarefaction makes individuals (and experimental animals) prone to develop salt-sensitive hypertension [33,34]. Contrary to PTC/tubule, GV was not associated with pre-donation $\Delta mGFR_{dopa}$. It is known that glomerular enlargement is accompanied by an increase in single-nephron GFR [1,2], which is also demonstrated in our study by a positive association between GV and pre-donation mGFR. We expected that an increase in GV would result in smaller $\Delta mGFR_{dopa}$, but this was not seen in our cohort. Power could be an issue here or maybe this association does not exist in a healthy population. In multivariable analysis GV and PTC/tubule had an additive effect on $\Delta mGFR_{dopa}$, suggesting

that their effects are partially independent. It might be that in individuals with larger GV, PTC/tubule provides information on the efficacy of the tubuloglomerular feedback mechanism after “renal stress”.

It has been thought that glomerular enlargement, i.e. hypertrophy, is a compensatory mechanism in response to an increased metabolic or hemodynamic demand and that over time it could lead to glomerulosclerosis, proteinuria and kidney function decline [35–37]. Consistent with this theory and in line with previous literature, the current study shows a positive and significant association of GV with blood pressure, waist/hip-ratio and BSA and borderline significant with BMI, all established risk factors of CKD (i.e. nephron loss) [38–40]. In a large U.S. cohort, GV is associated with a post-donation mGFR <60 mL/min/1.73m^{2,} [20], and with a ten-year post-donation mGFR <45 mL/min/1.73m^2 (but not <60 mL/min/1.73m^2) [12]. However, our study showed that larger GV was positively associated with three months- and five year post-donation mGFR. When comparing the characteristics of our donors to the aforementioned studies, the contrary results could possibly (partly) be explained by the seemingly higher BMI and lower pre-donation eGFR in the U.S. cohort (Mayo Clinic) compared to our cohort, which are both risk factors for lower post-donation kidney function [12]. In addition, glomerular density seemed higher in our cohort, compared to the U.S [14,41]. Possibly, there was a lower number of nephrons in individuals in the U.S. cohort, whereas in our cohort glomerular enlargement may have remained within physiological ranges. Physiological enlargement of glomeruli is supported by Lenihan et al. who postulated that glomerular hypertrophy post-donation is probably attributable to an increase in the glomerular ultrafiltration coefficient (K_f) and not to glomerular hypertension [5]. Moreover, recent findings in our cohort showed that a stronger short-term increase in post-donation single-kidney GFR, possibly accompanied by glomerular enlargement, predicted better five- and ten-year post-donation GFR [42]. Another reason for the contradictory results could be that kidney function impairment resulting from glomerular hypertrophy was not captured by the follow-up time in our cohort. More studies with greater sample size and follow-up beyond five years are warranted to clarify these discrepancies.

Strengths of this study include the precise kidney function measurements, and the presence of dopamine related renal function. Furthermore, our study is the first to study PTC density in relation to glomerular morphology and kidney function in healthy individuals. Although we did a power calculation, our study consisted of a small sample size, increasing the risk of missing effects due to limited

power. Secondly, we used biopsies taken from living donors before surgery, during surgery and/or after surgery (respectively T1, T2 and/or T3 biopsies). We cannot exclude that the surgical procedure affects PTC density, although in living donors with only little ischemic damage this effect is deemed small [3]. Furthermore, biopsies of different regions of the kidney may have been taken; however, Denic et al found that clinical characteristics show similar associations with glomerulosclerosis and GV at different cortical depths [36]. In addition, we found similar associations of glomerular morphology with clinical characteristics as previous studies, supporting the validity of our biopsies. Finally, the majority of our donors were Caucasian, making conclusions not generalizable to other ethnicities.

In conclusion, we found no association of PTC density with clinical characteristics or pre- and post-donation measured GFR, while GV is associated with pre-donation blood pressure, body size measurements and GFR. Measurement of PTC density may not provide prognostic information on kidney function after living kidney donation. Our findings support that glomerular and tubular enlargement in healthy kidneys may not be accompanied by an increase in peritubular capillaries. The association of the ratio between peritubular capillaries and tubules with kidney function after dopamine infusion may provide information on hemodynamic response mechanisms and warrants further investigation. Lastly, the relationship between peritubular capillaries and glomerular and tubular parameters in the preservation of renal function merits further study in health and disease.

STATEMENTS AND DECLARATIONS

Competing interests and funding
The authors declare no conflicts of interest.

Data availability statement
The datasets generated during and/or analysed during the current study are not publicly available due to privacy of the research participants but are available from the corresponding author on reasonable request.

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SUPPLEMENTARY MATERIAL

Table S1. Characteristics of the pre- and post-donation kidney function parameters.

Variable	Before donation	3 months post-donation	5 years post-donation
N	69	69	53
mGFR, mL/min	119±22	75±14	82±15
$mGFR_{dopa}$, mL/min	127±20	75±13	n.a.
$\Delta mGFR_{dopa}$, mL/min*	9±7	n.a.	n.a.

*calculated as $mGFR_{dopa}$ – mGFR.
Abbreviations: mGFR: measured glomerular filtration rate; $_{dopa}$: under stimulation of dopamine; n.a.: not applicable

Table S2. Characteristics of the population according to IF/TA percentage.

Variable	IF/TA>5%	IF/TA<5%	P value
N	15	54	-
Age, years	59±8	49±10	0.002
Sex, N (%} female	5 (33)	26 (48)	0.31
Race, N (%) Caucasian	16 (100)	56 (100)	-
BMI, kg/m^2	27±5	26±3	0.37
BSA, m^2	1.96±0.20	1.97±0.16	0.86
Waist/hip-ratio	0.99±0.10	0.94±0.07	0.08
SBP, mmHg	134±16	129±15	0.24
Serum HbA1c, %	5.9±1.2	5.6±0.5	0.16
Serum creatinine, mmol/L	76±15	80±12	0.35
Smoking, N (%) smokers	6 (40)	17 (31)	0.54
mGFR, mL/min	119±21	119±22	0.87
$mGFR_{dopa}$, mL/min	126±22	127±20	0.88
$\Delta mGFR_{dopa}$, mL/min	8±5	10±7	0.35
eGFR, mL/min/1.73m^2	89±10	87±14	0.76
Glomerular volume	0.0027±0.0009	0.0023±0.0006	0.09
PTC/tub	2.1±0.3	2.0±0.3	0.15
Tubular area	312278±10354	25104±8893	0.01
PTC/50.000m^2	25±4	27±4	0.20

Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; mGFR: measured glomerular filtration rate; $_{dopa}$: under stimulation of dopamine; eGFR: estimated glomerular filtration rate; PTC/tubule: peritubular capillary per tubule; PTC/50.000µm^2: peritubular capillary per 50.000µm^2.

Table S3. Multivariable linear regression analysis of glomerular volume, PTC/tubule and age with pre-donation mGFR.

Independent variables	Outcome	St.B	P	R^2
Glomerular volume	Pre-donation mGFR	0.29	0.01	0.22
PTC/tubule		0.13	0.26	
Age		-0.42	<0.001	
Glomerular volume	Pre-donation $mGFR_{dopa}$	0.25	0.02	0.29
PTC/tubule		0.29	0.01	
Age		-0.43	<0.001	
Glomerular volume	Pre-donation $\Delta mGFR_{dopa}$	-0.17	0.17	0.07
PTC/tubule		0.28	0.03	
Age		-0.17	0.17	

Green: P<0.05, orange: P 0.05 – 0.10
$\Delta mGFR_{dopa}$ =GFR_{dopa} – GFR (=renal functional reserve)

Table S4. Multivariable linear regression analysis of PTC/tubule, tubular area and age with pre-donation mGFR.

Independent variables	Outcome	St.B	P	R^2
PTC/tubule	Pre-donation mGFR	0.15	0.22	0.15
Tubular area		0.10	0.39	
Age		-0.41	<0.001	
PTC/tubule	Pre-donation $mGFR_{dopa}$	0.29	0.01	0.24
Tubular area		0.14	0.24	
Age		-0.43	<0.001	
PTC/tubule	Pre-donation $\Delta mGFR_{dopa}$	0.26	0.045	0.04
Tubular area		-0.03	0.83	
Age		0.18	0.16	

Green: P<0.05, orange: P 0.05 – 0.10
$\Delta mGFR_{dopa}$ =GFR_{dopa} – GFR (=renal functional reserve)

PART

C

8

CHAPTER

Early increase in single-kidney glomerular filtration rate after living kidney donation predicts long-term kidney function

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KIDNEY INTERNATIONAL (2022)

ABSTRACT

Single-kidney glomerular filtration rate (GFR) increases after living kidney donation due to compensatory hyperfiltration and structural changes. The implications of inter-individual variability in this increase in single-kidney GFR are unknown. Here, we aimed to identify determinants of the increase in single-kidney GFR at three-month postdonation, and to investigate its relationship with long-term kidney function. In a cohort study in 1024 donors, we found considerable inter-individual variability of the early increase in remaining single-kidney estimated GFR (eGFR) (median [25th-75th percentile]) 12 [8-18] mL/min/1.73m^2. Predonation eGFR, age, and cortical kidney volume measured by CT were the main determinants of the early postdonation increase in single-kidney eGFR. Individuals with a stronger early increase in single-kidney eGFR had a significantly higher five-year postdonation eGFR, independent of predonation eGFR and age. Addition of the postdonation increase in single-kidney eGFR to a model including predonation eGFR and age significantly improved prediction of a five-year postdonation eGFR under 50 mL/min/1.73m^2. Results at ten-year follow-up were comparable, while accounting for left-right differences in kidney volume did not materially change the results. Internal validation using ^{125}I-iothalamate-based measured GFR in 529 donors and external validation using eGFR data in 647 donors yielded highly similar results. Thus, individuals with a more pronounced increase in single-kidney GFR had better long-term kidney function, independent of predonation GFR and age. Hence, the early postdonation increase in single-kidney GFR, considered indicative for kidney reserve capacity, may have additional value to eGFR and age to personalize follow-up intensity after living kidney donation.

INTRODUCTION

In 1930, Ernest Basil Verney postulated that the kidney has reserve forces, a dormant renal reserve intended to cope with extraordinary hemodynamic and metabolic demands.[1] In line with this concept, donor nephrectomy is followed by an adaptive increase in glomerular filtration rate (GFR) by ~30% in the remaining kidney.[2,3,4] Although little is known about underlying mechanisms and determinants, early hemodynamic changes and structural adaptations of the remaining nephrons are generally considered to explain this increase.[5] A recent study identified a low nephron number for age as a predictor for long-term risk of chronic kidney disease after living kidney donation.[6] Whether reduced nephron number or cortical kidney volume, which has also been linked with postdonation kidney function,[7,8] are associated with a less pronounced postdonation increase in single-kidney GFR is unknown.

Interestingly, the magnitude of the postdonation increase in single-kidney GFR varies between individuals.[9] Hypothetically, an increased single-kidney GFR could lead to glomerular hypertension, glomerular injury, and loss of kidney function on the long term.[10,11] In contrast, a prior study showed that postdonation hyperfiltration by the remaining kidney is maintained stable by a combination of an increase in kidney plasma flow and in the ultrafiltration coefficient resulting from compensatory glomerular hypertrophy, not glomerular hypertension.[5] So far, the impact of the postdonation increase in single-kidney GFR on long-term postdonation kidney function remains unknown.

At the same time, the optimal estimation of long-term kidney function at living donor screening is key.[12,13] Previous studies identified age and predonation GFR as major predictors of long-term postdonation kidney function, although these factors together explained only 53% of the variation in postdonation GFR.[9] At least part of the unexplained variability may be accounted for by the postdonation increase in single-kidney GFR.

Therefore, in the present study, we hypothesized that the early postdonation increase in single-kidney GFR predicts long-term postdonation kidney outcomes. We first aimed to identify predonation determinants of this increase and subsequently investigated its capacity to predict long-term kidney function after donation.

METHODS

Study design and participants

An overview of participants and available data is provided in **Figure 1** and **Supplementary Table S1**. Data were used from all adults who donated a kidney between 1984 and 2018 at the University Medical Center Groningen, The Netherlands, and who provided informed consent. Both estimated GFR (eGFR) and measured (mGFR) data (details below) were available for the predonation screening visit and at 3 months postdonation in 1024 donors. Five- and 10-year follow-up of eGFR was available in 693 and 321 donors, respectively. Measured GFR, used for the internal validation of the analyses, was available in 529 and 236 donors at 5 and 10 years postdonation, respectively. The study was approved by the institutional ethical review board (2014/077) and was registered at clinicaltrials.gov under identifier NCT0327284.[14] An independent living kidney donor cohort from the Erasmus Medical Center (Rotterdam, The Netherlands) was used as a replication cohort. Design and study population of this cohort, consisting of 647 donors, are described in **Supplementary Methods**. All procedures were conducted in accordance with the Declaration of Helsinki, the Declaration of Istanbul, and the Dutch Scientific Guidelines.

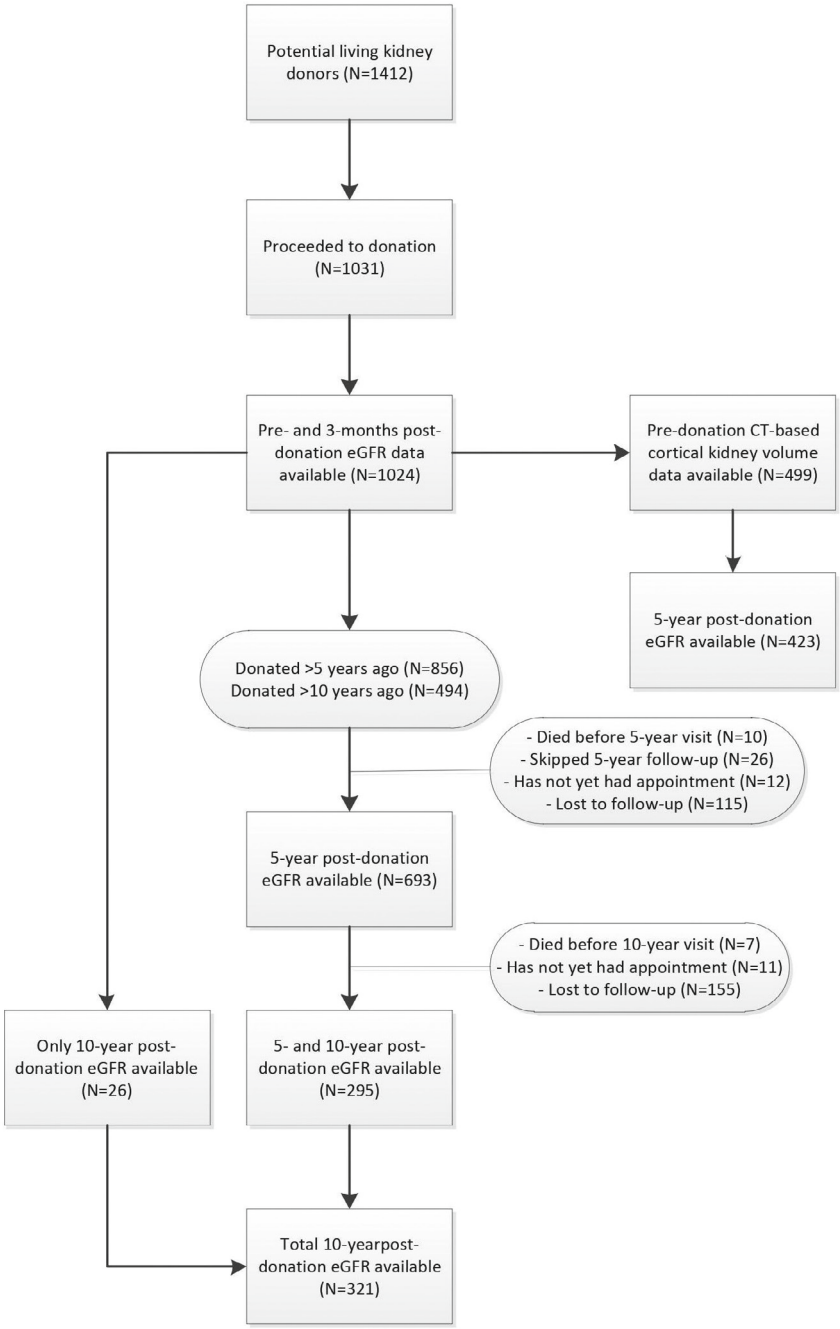

Figure 1. Overview of the study design and numbers of donors with available data.

CT, computed tomography; eGFR, estimated glomerular filtration rate.

Measurements and calculations

Primary analyses involved the eGFR using the (isotope dilution mass spectrometry–traceable) creatinine-based Chronic Kidney Disease Epidemiology Collaboration formula.[15] Internal validation was performed in a subgroup of donors with mGFR data by using radiolabeled iothalamate (^{125}I-iothalamate) clearance, as described in detail previously.[16] Details on serum creatinine and mGFR measurements are provided in **Supplementary Methods**. The day-to-day variability of ^{125}I-iothalamate–based mGFR is 2.5%.[16,17] The postdonation increase in single-kidney GFR was calculated as the GFR at 3 months postdonation minus 50% of the predonation GFR.[18] To account for left-right differences in kidney volume, we performed a secondary analysis where we recalculated the postdonation increase in single-kidney GFR by using the remaining kidney volume as a percentage of the total volume of both kidneys using computerized tomography (CT). Preoperative kidney CT obtained during the corticomedullary phase (scanned 20–25 seconds after i.v. contrast injection) was automatically segmented; the volumes of the kidney cortex and medulla of both kidneys were calculated separately, and cortical kidney volume of the remaining (nondonated) kidney was used for further analysis. The CT scans were performed routinely in every donor between 2007 and 2016 and were therefore available only in a subgroup of donors.

Other clinical and biochemical measurements were performed as described previously.[14] Diabetes was diagnosed according to the American Diabetes Association criteria.[19] Proteinuria was determined using the protein-creatinine ratio in a spot urine sample.[20]

Statistical analyses

Data are presented as mean ± SD for normally distributed variables and as median (25th–75th percentile) for nonnormally distributed variables. Binary variables are shown as number (percentage). The distribution was tested using histograms and probability plots. The characteristics of the population are presented for the whole cohort and according to tertiles of the postdonation increase in single-kidney GFR. In cross-sectional analyses, we aimed to identify independent predonation determinants of the short-term postdonation increase in single-kidney GFR, including all potential determinants of this parameter.[3,9,21] Variables with univariable P values <0.05 were subsequently included in a multivariable linear regression model. Because we hypothesized that the cortical volume of the remaining kidney would be a major determinant, we performed similar analyses in a subgroup with available CT-based kidney volume data.

Next, in longitudinal analyses, we used multivariable linear regression models to investigate the association between the (short-term) postdonation increase in single-kidney GFR and the eGFR at 5 and 10 years postdonation. We similarly used linear regression analysis to study the associations between the postdonation increase in single-kidney GFR and the development of proteinuria at 5 and 10 years postdonation. Models were adjusted for predonation eGFR and donor age as well-established determinants of long-term postdonation kidney function.[9] Multicollinearity was examined in all models using the variance inflation factor; only variables with a variance inflation factor of <3 were included in the models.

We subsequently assessed the capacity of the postdonation increase in single-kidney GFR to predict an eGFR of <50 ml/min per 1.73 m^2 at 5 and 10 years postdonation beyond established predictors (i.e., age and predonation eGFR). This threshold was selected to make sure that >10% of donors would reach the end point, allowing for reliable risk prediction. We compared the performance of basic models including GFR and age with or without the postdonation increase in single-kidney GFR by using receiver operating characteristic curve analyses to calculate the area under the curve (AUC) for predicting an eGFR of <50 ml/min per 1.73 m^2 at 5 and 10 years postdonation. Differences in AUCs between models were tested according to DeLong et al.[22] In addition, we calculated the net reclassification improvement and the integrated discrimination improvement index by comparing 2 logistic regression models for the risk of reaching a 5- and 10-year postdonation eGFR of <50 ml/min per 1.73 m2.[23,24] We performed several sensitivity analyses including replication of analyses using the mGFR-based postdonation increase in single-kidney GFR in the same cohort and external validation in a cohort with pre- and postdonation eGFR (see Supplementary Methods).

SPSS version 23 for Windows (IBM Corporation), RStudio version 1.1.463, and GraphPad Prism 6 for Windows (GraphPad) were used to perform the analyses. P values <0.05 were considered statistically significant.

RESULTS

Characteristics of the donor cohort

The characteristics of the study participants are summarized in **Table 1**. At the predonation screening visit, donors were 52 ± 11 years old, 52% were female, and all donors were White. The mean eGFR was 91 ± 15 ml/min per 1.73 m^2

predonation, 59 ± 13 ml/min per 1.73 m^2 at 3 months postdonation (P < 0.001 vs. predonation), and 62 ± 13 and 63 ± 13 ml/min per 1.73 m^2 at 5 and 10 years postdonation (for both, P < 0.001 vs. 3 months postdonation). The median (25th–75th percentile) increase in eGFR beyond 50% of the predonation eGFR at 3 months postdonation was 12 (8–18) ml/min per 1.73 m^2.

Determinants of postdonation increase in single-kidney eGFR

In univariable analyses, age, body mass index, systolic blood pressure, HbA1c, and body surface area were inversely associated and predonation eGFR was positively associated with the postdonation increase in single-kidney eGFR (**Table 2**). In the final multivariable model, predonation eGFR, age, and body surface area were independent determinants (**Table 2**). A secondary analysis in a subgroup of donors with available CT-based cortical kidney volume data (n = 499; characteristics in **Supplementary Table S2**) identified cortical kidney volume as another independent determinant (**Supplementary Table S3**).

Postdonation increase in single-kidney eGFR and long-term kidney function

Five- and 10-year eGFR follow-up was available for 693 and 321 donors, respectively; the predonation characteristics of these subgroups are provided in **Supplementary Table S4** and were highly similar to the full cohort (**Table 1**). Five- and 10 years postdonation eGFR values according to postdonation increase in single-kidney eGFR tertiles are provided in **Figure 2**. The postdonation increase in single-kidney eGFR was associated with eGFR at 5 years after donation both in univariable analysis (**Supplementary Table S5**) and after adjustment for predonation eGFR and age (St. β = 0.33; P < 0.001; Table 3). Adding the postdonation increase in single-kidney eGFR to a model with predonation eGFR and age significantly improved the model R^2 (0.58–0.68; P < 0.001; **Table 3**). Similar results were obtained in the subgroup with data available on 10-year postdonation follow-up (**Table 3**). The postdonation increase in single-kidney eGFR was not associated with a protein-creatinine ratio of >15 mg/mmol at 5 and 10 years postdonation (odds ratio 1.02; 95% confidence interval [CI] 0.99–1.04; P = 0.21, n = 650 and odds ratio 1.01; 95% CI 0.98–1.05; P = 0.49, n = 301, respectively; **Supplementary Table S6**).

Table 1. Characteristics of living kidney donors according to tertiles of post-donation increase in single-kidney eGFR (Δsk-eGFR).

		Δsk-eGFR		
	Total	**Low**	**Intermediate**	**High**
		<10 mL/min	**10-16 mL/min**	**>16 mL/min**
Number	1024	341	341	342
Female sex	531 [52]	160 [47]	189 [55]	182 [53]
Caucasian race	1028 [100]	343 [100]	343 [100]	342 [100]
Age, years	52 (11)	56 (10)	52 (10)	47 (11)
Weight, kg	80 (14)	82 (14)	80 (14)	78 (13)
Height, cm	175 (9)	175 (10)	175 (9)	175 (10)
BMI, kg/m^2	26 (4)	27 (3)	26 (3)	26 (4)
BSA, m^2	1.95 (0.20)	1.97 (0.20)	1.95 (0.19)	1.93 (0.19)
SBP, mmHg	126 (13)	128 (13)	127 (13)	124 (12)
DBP, mmHg	76 (9)	76 (9)	76 (9)	75 (9)
Hypertension*	159 [16]	62 [18]	49 [14]	48 [14]
Use of antihypertensives	142 [14]	64 [19]	39 [11]	39 [11]
ACE inhibitors	54 [5]	25 [7]	10 [3]	19 [6]
ARBs	30 [3]	16 [5]	11 [3]	3 [1]
Betablockers	53 [5]	21 [6]	15 [4]	17 [5]
Calcium antagonists	27 [3]	12 [4]	8 [2]	7 [2]
Diuretics	43 [4]	20 [6]	10 [3]	13 [4]
Statins	39 [4]	16 [5]	14 [4]	9 [3]
Pre-donation eGFR, mL/min/1.73m^2	91 (15)	86 (16)	90 (12)	96 (14)
Pre-donation mGFR, mL/min	114 (21)	108 (20)	113 (21)	120 (22)
Serum creatinine, µmol/L	75 (13)	79 (13)	75 (12)	72 (13)
Serum glucose, mmol/L	5.3 (0.6)	5.4 (0.7)	5.3 (0.5)	5.2 (0.5)
HbA1C, %	5.5 (0.4)	5.5 (0.4)	5.5 (0.4)	5.4 (0.4)
Current smoking	249 [24]	61 [18]	78 [23]	110 [32]
Serum cholesterol, mmol/L	5.3 (1.0)	5.4 (1.0)	5.4 (1.0)	5.2 (1.1)
LDL	3.5 (0.9)	3.5 (0.9)	3.5 (1.1)	3.4 (0.9)
HDL	1.5 (0.5)	1.4 (0.4)	1.7 (0.5)	1.6 (0.6)
Triglycerides	1.4 (0.9)	1.4 (0.8)	1.4 (0.9)	1.3 (0.9)
Serum urea, mmol/L	5.4 (1.3)	5.8 (1.3)	5.4 (1.2)	5.1 (1.4)
Serum potassium, mmol/L	4.0 (0.3)	3.9 (0.3)	3.9 (0.3)	4.0 (0.4)
Serum sodium, mmol/L	141 (3)	141 (2)	141 (2)	140 (3)
Sodium excretion, mmol/24h	194 (74)	196 (74)	196 (75)	190 (74)

Data presented as mean (standard deviation) or n [%].

*SBP >140 mmHg and/or DBP >90 mmHg

Abbreviations: ACE inhibitors: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers;BMI: body mass index; BSA: body surface area; Δsk-eGFR: post-donation increase in single-kidney eGFR; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate; SBP: systolic blood pressure.

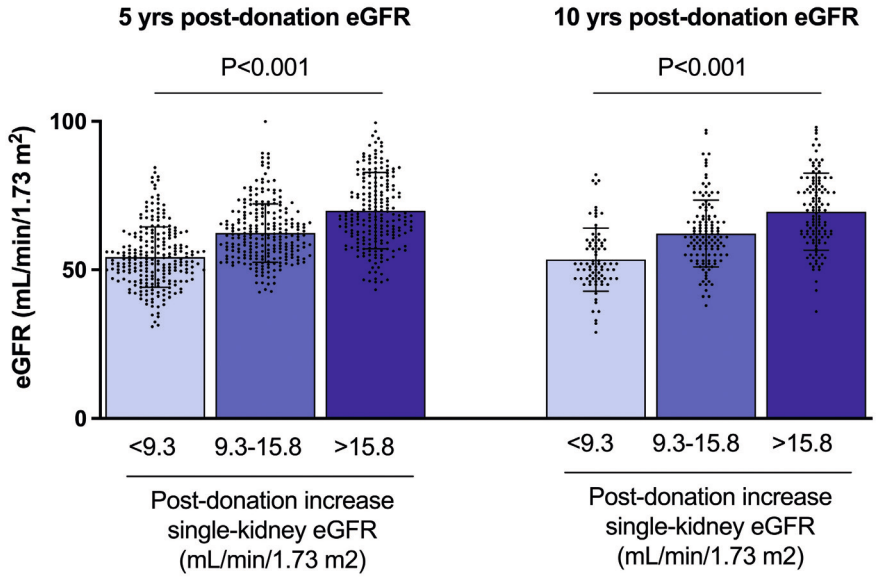

Figure 2. Five- and 10-year postdonation estimated glomerular filtration rate (eGFR) according to tertiles of the early postdonation increase in single-kidney eGFR.

Bars and error bars indicate means and SDs.

Table 2. Pre-donation determinants of post-donation increase in single-kidney GFR.

	Univariable		Multivariable	
	St. β	P	St. β	P
eGFR	0.34	<0.001	0.22	<0.001
Age	-0.33	<0.001	-0.23	<0.001
BMI	-0.11	0.001	-	-
SBP	-0.10	0.001	-	-
BSA	-0.09	0.003	-0.13	<0.001
HbA1C	-0.08	0.02	-	-
Female sex	0.05	0.15	-	-
Sodium excretion	0.05	0.13	-	-

Multivariable model R^2= 0.16

Abbreviations: BMI: body mass index; BSA: body surface area; CI: confidence interval; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; St. β: standardized beta.

We next investigated the capacity of the postdonation increase in single-kidney eGFR to improve the prediction of an eGFR of <50 ml/min per 1.73 m^2 at 5 and 10 years postdonation. Of the 693 donors with 5-year postdonation eGFR data

available, 108 reached an eGFR of <50 ml/min per 1.73 m^2 at 5 years post-donation (median [range] 46 [31–49] ml/min per 1.73 m^2). Receiver operating characteristic curve analyses demonstrated that predonation eGFR combined with age (model 1, **Table 3**) strongly predicted a 5-year eGFR of <50 ml/min per 1.73 m^2 (AUC 89%; 95% CI 86%–92%). Addition of the postdonation increase in single-kidney eGFR (model 2, **Table 3**) improved prediction (AUC 92%; 95% CI 90%–94%; P = 0.01 vs. model 1). Addition of the postdonation increase in single-kidney eGFR to a logistic regression model that also included predonation eGFR and age improved the reclassification of donors who reached a 5-year eGFR of <50 ml/min per 1.73 m^2 (integrated discrimination improvement 0.08; net reclassification improvement 0.16; P < 0.001; **Table 4.**

Table 3. Multivariable associations of post-donation increase in single-kidney GFR with long-term post-donation eGFR in the main cohort and independent validation cohort.

	Main cohort				Independent validation cohort			
	St. β	**P**	**R^2**	**R^2 change**	**St. β**	**P**	**R^2**	**R^2 change**
5-year eGFR (N=693)					*5-year eGFR (N=647)*			
Model 1								
eGFR	0.67	<0.001	0.58		0.67	<0.001	0.62	
Age	-0.16	<0.001			-0.16	<0.001		
Model 2								
eGFR	0.59	<0.001			0.59	<0.001		
Age	-0.10	0.001	0.68	<0.001	-0.11	<0.001	0.70	<0.001
Dsk-eGFR	0.33	<0.001			0.30	<0.001		
10- year eGFR (N=321)								
Model 1								
eGFR	0.67	<0.001	0.45					
Model 2								
eGFR	0.57	<0.001	0.55	<0.001				
Δsk-eGFR	0.34	<0.001						

Age, eGFR and BSA were measured pre-donation. Age was not an independent determinant of ten-year post-donation eGFR and was therefore withdrawn from the ten-year post-donation eGFR model. The independent validation cohort had no 10-year eGFR data available.

Abbreviations: BSA: body surface area; Dsk-eGFR: post-donation increase in single-kidney eGFR; eGFR: estimated glomerular filtration rate; St. β: standardized beta.

Table 4. Reclassification table of models with vs. without the post-donation increase in single-kidney GFR for predicting a five or ten-year post-donation eGFR <50 mL/min/1.73m².

Without post-donation increase in single-kidney GFR	With post-donation increase in single-kidney GFR				%correctly reclassified		
	<30%	30-60%	>60%	Total (n)	increased risk	decreased risk	Net correctly reclassified
Risk of five year post-donation eGFR <50 mL/min/1.73m²							
Donors reaching a 5-yr post-donation eGFR <50 mL/min/1.73m²					25%	9%	16%
<30%	22	12[a]	2[a]	36			
30-60%	7[b]	16	13[a]	36			
>60%	0[b]	3[b]	33	36			
Total	29	31	48	108			
Donors not reaching a 5-yr post-donation eGFR <50 mL/min/1.73m²					4%	4%	0%
<30%	507	19[b]	1[b]	527			
30-60%	17[a]	23	4[b]	44			
>60%	0[a]	6[a]	8	14			
Total	524	48	13	585			
Risk of ten year post-donation eGFR <50 mL/min/1.73m²							
Donors reaching a 10-yr post-donation eGFR <50 mL/min/1.73m²					42%	8%	34%
<30%	13	6[a]	6[a]	25			
30-60%	0[b]	8	9[a]	17			
>60%	0[b]	4[b]	4	8			
Total	13	18	19	50			
Donors not reaching a 10-yr post-donation eGFR <50 mL/min/1.73m²					7%	4%	-3%

Table 4. Reclassification table of models with vs. without the post-donation increase in single-kidney GFR for predicting a five or ten-year post-donation eGFR <50 mL/min/1.73m^2. (continued)

Without post-donation increase in single-kidney GFR	With post-donation increase in single-kidney GFR				%correctly reclassified		
	<30%	30-60%	>60%	Total (n)	increased risk	decreased risk	Net correctly reclassified
Risk of five year post-donation eGFR <50 mL/min/1.73m^2							
Donors reaching a 5-yr post-donation eGFR <50 mL/min/1.73m^2					25%	9%	16%
<30%	234	14[b]	2[b]	250			
30-60%	11[a]	4	2[b]	17			
>60%	0[a]	1[a]	3	4			
Total	245	19	7	271			

Model predicting five year post-donation eGFR <50 mL/min/1.73m^2
- Net reclassification index: 0.16 (P<0.001)
- Integrated discrimidation improvement: 0.08 (P<0.001)

Model predicting ten year post-donation eGFR <50 mL/min/1.73m^2
- Net reclassification index: 0.32 (P<0.001)
- Integrated discrimidation improvement: 0.18 (P<0.001)

[a]Donors who were correctly reclassified by the model including the post-donation increase in single-kidney GFR

[b]Donors who were incorrectly reclassified by the model including the post-donation increase in single-kidney GFR

Abbreviations: eGFR: estimated glomerular filtration rate.

Of the 321 donors with 10-year postdonation eGFR data available, 50 reached a 10-year postdonation eGFR of <50 ml/min per 1.73 m^2 (median [range] 46 [29–49] ml/min per 1.73 m^2). Predonation eGFR (model 1, **Table 3**) predicted a 10-year postdonation eGFR of <50 ml/min per 1.73 m^2 with an AUC of 81% (95% CI 75%–87%). Addition of the postdonation increase in single-kidney eGFR (model 2, **Table 3**) increased the AUC to 88% (95% CI 83%–94%; P < 0.001 vs. model 1). The postdonation increase in single-kidney eGFR also improved the reclassification of donors who reached a 10-year postdonation eGFR of <50 ml/min per 1.73 m^2 (integrated discrimination improvement 0.18; net reclassification improvement 0.32; P < 0.001; **Table 4**).

Sensitivity analyses and internal and external validation

We based our definition of the postdonation increase in single-kidney GFR on the assumption that 50% of kidney mass remains after donation. In a sensitivity analysis, we changed the definition to account for the actual percentage of remaining kidney volume (see **Supplementary Methods**). The modified postdonation increase in single-kidney eGFR was still associated with 5- and 10-year eGFR (**Supplementary Table S7**). Next, we internally validated our longitudinal analyses by redefining the postdonation increase in single-kidney GFR based on mGFR (^{125}I-iothalamate), yielding similar results regarding the associations with 5- and 10-year mGFR (**Supplementary Table S8**). Then, we repeated all analyses using the eGFR-based postdonation increase in single-kidney eGFR in an independent validation cohort of 647 donors with 5-year postdonation eGFR available (characteristics in **Supplementary Table S9**). Multivariable linear regression also revealed age and predonation eGFR as main determinants of the postdonation increase in single-kidney eGFR in the validation cohort (**Supplementary Table S10**). The postdonation increase in single-kidney eGFR was similarly associated with eGFR at 5 years in the validation cohort (**Table 3**). Lastly, we calculated the postdonation increase in single-kidney eGFR based on the postdonation eGFR as a percentage of the predonation eGFR. This relative postdonation increase in single-kidney GFR was also associated with long-term eGFR (**Supplementary Table S11**).

Practical implications

To illustrate the implications of the postdonation increase in single-kidney GFR beyond predonation and early postdonation GFR, 2 sets of examples are presented in **Figure 3**. Figure 3a shows the eGFR course for 2 individual patients with an identical predonation eGFR but with different early postdonation eGFR values and subsequently with different eGFR values at 5 years postdonation.

Figure 3b shows 2 individual patients with different predonation eGFR values but with an identical eGFR at 3 months postdonation. These patients therefore also had different early increases in single-kidney eGFR and had different long-term eGFR values.

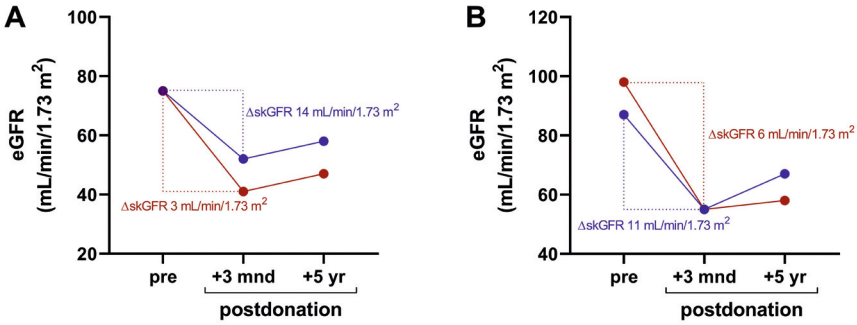

Figure 3. Examples illustrating application of the postdonation increase in single-kidney estimated glomerular filtration rate (eGFR).

(a) Two individual patients with identical predonation eGFR (75 ml/min per 1.73 m^2). One patient (blue symbols) had a higher early postdonation eGFR (52 ml/min per 1.73 m^2), resulting from a relatively stronger increase in single-kidney eGFR [Δsk-eGFR = 52 – (75/2) = 14 ml/min per 1.73 m^2]. This patient had a higher long-term eGFR (57 ml/min per 1.73 m^2). The other patient (red symbols) had a lower early postdonation eGFR (41 ml/min per 1.73 m^2), as a result of a less pronounced increase in single-kidney eGFR [Δsk-eGFR = 41 – (75/2) = 3 ml/min per 1.73 m^2]. This patient, despite the identical early postdonation eGFR, had a lower long-term eGFR (49 ml/min per 1.73 m^2). (b) Two individual patients with identical eGFR at 3 months postdonation (55 ml/min per 1.73 m^2). One patient (blue symbols) had a lower predonation eGFR (87 ml/min per 1.73 m^2), resulting in a relatively stronger increase in single-kidney eGFR [Δsk-eGFR = 55 – (87/2) = 11 ml/min per 1.73 m^2]. This patient had a higher long-term eGFR (67 ml/min per 1.73 m^2). The other patient (red symbols) had a higher predonation eGFR (98 ml/min per 1.73 m^2), resulting in a less pronounced increase in single-kidney eGFR [Δsk-eGFR = 55 – (98/2) = 6 ml/min per 1.73 m^2]. This patient, despite the identical early postdonation eGFR, had a lower long-term eGFR (58 ml/min per 1.73 m^2).

DISCUSSION

This study aimed to investigate the predictive value of short-term postdonation kidney function adaptation, defined as the postdonation increase in single-kidney GFR, for long-term postdonation GFR. Furthermore, we aimed to identify predonation determinants of the postdonation increase in single-kidney GFR. We found that the postdonation increase in single-kidney GFR improves the prediction of long-term postdonation kidney function beyond predonation mGFR

and age. Independent determinants of the postdonation increase in single-kidney GFR were age, predonation GFR, and cortical volume of the remaining kidney.

Prediction of long-term postdonation kidney function has been a major goal in transplant nephrology for decades,[25] and several stress tests have been developed to investigate the potential role of the renal reserve capacity in this context. We previously reported that dopamine-induced GFR stimulation before living kidney donation was associated with short-term but not long-term mGFR postdonation.[21] This suggests that predonation dopamine stimulation might reflect only hemodynamic processes that play a dominant role in the early postdonation GFR adaptation, but not long-term adaptation, which might be more a result of adaptive structural changes after kidney donation.[21] The same likely applies to oral/i.v. amino acid administration.[26] In current practice, predonation kidney function and age are often used to estimate postdonation kidney function.[12,13] This study shows that early postdonation GFR adaptation improves the prediction of long-term postdonation kidney function beyond the absolute values of pre- or postdonation GFR and might inform about the reserve capacity of the remaining kidney. In other words, donors with the same pre- or postdonation GFR but differences in postdonation increase in single-kidney GFR displayed differences in long-term kidney function, as shown in the longitudinal analyses of this study. This is in line with the conclusions of a previous study.[27] Possibly, an early increase in GFR reflects a more physiological mechanism of adaptation to acute reduction in kidney mass (i.e., a better renal functional reserve) whereas slow/long-term postdonation increase in GFR may reflect more structural or even pathophysiological changes in the kidney. Of interest, a recent study found that subclinical nephrosclerosis, larger cortical nephron size, and smaller medullary volume observed in intraoperative biopsies in healthy donors predicted recipient death-censored graft failure independently of donor or recipient clinical characteristics.[28] Moreover, another recent study from the same group established an association between nephron number and residual eGFR, defined as postdonation eGFR divided by predonation eGFR.[6] In support of an underlying relationship with residual kidney mass, our study showed that (remaining) kidney volume is an independent determinant of the postdonation increase in single-kidney GFR. The postdonation increase in single-kidney GFR could be used to guide the intensity of donor follow-up by identifying individuals at risk of decreased GFR on the longer term. The 2017 Kidney Disease: Improving Global Outcomes guideline states that for each donor a personalized plan for follow-up should be made, which describes who should perform follow-up care and how often. It is not specified how this should be personalized. Our study may be useful to

guide personalization, as donors with low postdonation increase in single-kidney GFR, similar to dose with low predonation eGFR, might benefit from extended follow-up in the transplant center.

Whether a relatively high postdonation GFR reflects renal reserve or hyperfiltration linked with poor outcome has been a long-standing debate. In various settings, such as diabetic nephropathy, decreased GFR impairment is preceded by hyperfiltration.[11,29] Landmark studies by Brenner and colleagues showed that hyperfiltration is followed by kidney damage and proteinuria in animals.[29,30] However, so far it has been unclear how these observations relate to unilateral nephrectomy in healthy donors. We found that a more pronounced postdonation increase in single-kidney GFR was associated with better, not decreased, long-term GFR, and we found no independent association with the development of proteinuria. Instead, donors with low postdonation increase in single-kidney GFR had worse outcomes on the long-term after donation, possibly because these donors already suffered from nephron loss before donation. Whether donors with a more pronounced postdonation increase in single-kidney GFR retain additional reserve in case of a postdonation "second hit" (e.g., new-onset diabetes) remains unknown.

Donor age and predonation GFR were the main determinants of the postdonation increase in single-kidney GFR, in line with a previous study by our group using the dopamine-based renal functional reserve.[3] The inverse association between age and the postdonation increase in single-kidney GFR could indicate aging-related subclinical kidney injury. Although we analyzed a wide range of variables and identified 3 independent determinants, the multivariable model explained only 16% of variance in the postdonation increase in single-kidney GFR ($R^2 = 0.16$, **Table 2**), limiting predonation applicability. The inverse association between systolic blood pressure and the postdonation increase in single-kidney GFR that was found in univariable analyses points toward the suggestion that donors with hypertension might suffer from nephron loss and therefore retain less capacity to increase GFR postdonation. However, this association lost significance after adjustment for age, which also applies to sex, body mass index, and HbA1c. The association with (cortical) kidney volume is in line with previous studies connecting kidney volume with (postdonation) GFR.[8,31] Our findings pave the way for future studies that identify underlying molecular mechanisms, define biomarkers, and reveal the clinical potential of the postdonation increase in single-kidney GFR, both in and beyond kidney donation.

Our main cohort is unique in that it consists of a large number of donors with both repeated eGFR and mGFR measurements before and up to 10 years after donation. Although we cannot fully exclude residual confounding, our results were robust upon multivariable adjustment and consistent in internal and external validation cohorts. Limitations of our study include poor generalizability to populations other than White people and the lack of follow-up beyond 10 years precluding conclusions on the potential impact on the risk of kidney failure. The time range in which the postdonation compensatory increase in kidney function is determined varies among studies, and in our center, only 3 months postdonation eGFR and mGFR data were available.[25] Lastly, the postdonation increase in single-kidney GFR cannot be assessed before donation, underlining the need to develop adequate biomarkers in addition to age, cortical kidney volume, and kidney function.

In conclusion, we found that the postdonation increase in single-kidney GFR predicts long-term kidney function independent of predonation GFR, age, and body surface area. Our findings provide novel insights in the prognostic potential of the kidney's reserve capacity.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

We thank T.M. Royaards, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, for her contribution to the data collection and management of the replication cohort.

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SUPPLEMENTARY MATERIAL

Supplementary Methods

Measurement of serum creatinine

Serum creatinine was measured routinely in our central chemistry laboratory by an isotope dilution mass spectrometry (IDMS) traceable enzymatic assay on the Roche Modular (Roche Ltd., Mannheim, Germany) from 1st March 2006 onwards. Before this date, samples were measured by Jaffe alkaline picrate assay on the Merck Mega Analyzer (Merck, Darmstadt, Germany). Values obtained by the Jaffe method were converted to allow comparison with the Roche method by the formula (Y^{Roche} =(X^{Jaffe} -8)/1.07) (S1). To ensure this conversion did not affect the results, we split the cohort based on serum creatinine measurement method and repeated several analyses in these separate groups:

Estimated GFR before and after 1rst March 2006	
Screening before 2006	
N	228
Mean ±SD eGFR	94 ±13
Screening after 2006	
N	800
Mean ±SD eGFR	90 ±15

mGFR measurements

The mGFR was calculated as clearance of radiolabeled iothalamate (^{125}I-iothalamate) (S2). Measurements were done in fasting condition, without prehydration or interruption of medication. Before constant infusion of iothalamate started, a blood sample was drawn from the donors. This blood sample was used for routine laboratory measurements. Subsequently, infusion of iothalamate at 0.04 ml/kg body weight was started. The infusion solution contained 0.04 MBq of ^{125}I-iothalamate (following an initial dose of 0.6 MBq ^{125}I-iothalamate) and 0.03 MBq ^{131}I-hippurate and was started at 8:00 a.m. at an infusion rate of 12 ml/h. After a stabilization period, measurements started at 10:00 a.m. Clearances were calculated as (U*V)/P and (I*V)/P, where U*V represents the urinary excretion, I*V represents the infusion rate of the tracer and P represents the plasma tracer concentration per clearance period. From clearance levels of these traces, GFR, effective renal plasma flow, and filtration fraction were calculated. Correction for incomplete bladder emptying and dead space was achieved by multiplying

the urinary ^{125}I-iothalamate clearances with plasma and urinary ^{131}I-hippurate clearance. The day-to-day variability of the mGFR is 2.5% (S3).

Increase in single-kidney mGFR

In primary analyses in the discovery cohort, the increase in single-kidney GFR was calculated using pre- and post-donation eGFR data. However, because eGFR underestimates mGFR (S4), we also used a reference method, mGFR (^{125}I-iothalamate) to calculate the post-donation increase in single-kidney mGFR (Dsk-mGFR) and repeated all analyses. The Dsk-mGFRwas calculated as the mGFR at 3 months post-donation minus 50% of the predonation mGFR.

Validation cohort

We subsequently studied the association between the post-donation increase in single-kidney GFR and five-year post-donation eGFR, and repeated these analyses in an external cohort of 647 living kidney donors from the Erasmus Medical Center Rotterdam, The Netherlands. The donors in the Rotterdam cohort donated between 1992 and 2013. Renal function (eGFR, CKD-EPI) and clinical parameters as height, weight, and blood pressure were measured before donation, three months after donation, and five years after donation.

Sensitivity analyses

Our RFR definition is based on the assumption that 50% of renal mass remains after donation. In a first sensitivity analysis, we changed the post-donation increase in single-kidney GFR calculation to account for the actual percentage of remaining kidney cortical volume, as follows:

$$\Delta sk\text{–}GFR = GFR\ 3m\ postdonation - \left(\frac{remaining\ kidney\ cortical\ volume}{[cortical\ volume\ left + right\ kidney]} \times predonation\ GFR\right)$$

We also calculated the post-donation increase in single-kidney GFR based on the post-donation eGFR as a percentage of the pre-donation eGFR.

Supplementary Table S1. Overview of available data.

	Pre-donation	3 months post-donation	5-years post-donation	10 years post-donation
N donors	1024	1024	856	494
N donors at follow-up visit	1024	1024	695	321
Serum creatinine/eGFR	1024	1024	695	321
Protein/creatinine ratio	815	Not used in analyses	650	301
mGFR	1024	1024	529	236
Cortical kidney volume	499	Not used in analyses	Not used in analyses	Not used in analyses

At five and ten years post-donation, mGFR data were missing for the following reasons (listed from most common to least common reason):
1) In 2016 and 2017, there was a temporary stop in mGFR measurements due to ^{125}I-iothalamate delivery issues
2) Donor declined mGFR measurement for unspecified/personal reasons
3) Unknown reason
4) mGFR measurement not performed due to COVID-19

Supplementary Table S2. Living kidney donor characteristics before donation in subgroup with and without CT-based (remaining) kidney cortical volume data available.

	Kidney volume available	Kidney volume not available
0	499	525
Female sex, n [%]	257 [52]	275 [52]
White race, n [%]	498 [100]	529 [100]
Age, years	53 (11)	51 (11)[a]
Weight, kg	80 (14)	80 (14)
Height, cm	175 (9)	175 (9)
BMI, kg/m^2	26 (3)	26 (4)
BSA, m^2	1.95 (0.20)	1.95 (0.20)
SBP, mmHg	127 (14)	126 (13)
DBP, mmHg	76 (9)	76 (9)
Hypertension*, n [%]	79 [16]	81 [15]

Supplementary Table S2. Living kidney donor characteristics before donation in subgroup with and without CT-based (remaining) kidney cortical volume data available. (continued)

	Kidney volume available	Kidney volume not available
Use of antihypertensives, n [%]	77 [15]	65 [12]
ACE inhibitors	29 [6]	25 [5]
ARBs	17 [3]	13 [3]
Betablockers	29 [6]	24 [5]
Calcium antagonists	15 [3]	12 [3]
Diuretics	24 [5]	19 [4]
Statins	16 [3]	23 [4]
eGFR, mL/min/1.73m^2	91 (16)	91 (13)
mGFR, mL/min	114 (22)	114 (21)
Δsk-eGFR, mL/min/1.73m^2	15 (9)	12 (8)[b]
Δsk-eGFR%, %	16 (10)	13 (8)[b]
Δsk-mGFR, mL/min	16 (9)	15 (7)
Kidney volume, mL	187 (37)	n.a.
Serum creatinine, μmol/L	75 (14)	76 (13)
Serum glucose, mmol/L	5.3 (0.6)	5.3 (0.6)
HbA1c, %	5.5 (0.4)	5.5 (0.4)
Serum cholesterol, mmol/L	5.3 (1.0)	5.3 (1.0)
LDL	3.5 (0.9)	3.4 (1.0)
HDL	1.5 (0.4)	1.5 (0.6)
Triglycerides	1.3 (0.8)	1.5 (0.9)[a]
Serum urea, mmol/L	5.5 (1.4)	5.3 (1.3)[a]
Serum potassium, mmol/L	3.9 (0.3)	4.0 (0.3)[b]
Serum sodium, mmol/L	142 (2)	140 (3)[b]
Sodium excretion, mmol/24h	200 (74)	186 (74)[a]

Data presented as mean (standard deviation) or n [%].

* SBP >140 mmHg and/or DBP >90 mmHg

[a]: P<0.05, [b]: P<0.001

Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; ACE inhibitors: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate; Δsk-eGFR: post-donation increase in single-kidney eGFR; Δsk-eGFR%: relative post-donation increase in single-kidney GFR; Δsk-mGFR: post-donation increase in single-kidney measured GFR; LDL: low-density lipoprotein; high-density lipoprotein.

Supplementary Table S3. Determinants of the post-donation increase in single-kidney estimated glomerular filtration rate (eGFR) in a subgroup with remaining kidney cortical volume data available (N=499).

	Univariable		Multivariable	
	St. β	P	St. β	P
eGFR	0.42	<0.001	0.36	<0.001
Age	-0.29	<0.001	-	-
Cortical kidney volume	0.25	<0.001	0.13	0.003
SBP	-0.14	0.002	-	-
HbA1C	-0.11	0.01	-	-
BMI	-0.10	0.02	-0.12	0.01
BSA	-0.03	0.54	-	-
Sex	-0.01	0.83	-	-
Sodium excretion	-0.04	0.35	-	-

Multivariable model R2= 0.20
Data obtained by multivariable linear regression using eGFR, age, and remaining kidney volume at three months before donation.
Abbreviations: eGFR: estimated glomerular filtration rate; St. β: standardized beta; SBP: systolic blood pressure; BMI: body mass index; BSA: body surface area.

Supplementary Table S4. Living kidney donor characteristics before donation in subgroups with and without five- and ten-year post-donation eGFR data available.

	Donated >5 year ago (N=856)		Donated >10 years ago (N=494)	
Baseline parameters	*5 year follow-up available*	*5 year follow-up not available*	*10 year follow-up available*	*10 year follow-up not available*
Number, n	693	163	321	173
Female sex, n [%]	365 [53]	88 [52]	173 [54]	93 [53]
White race, n [%]	518 [100]	163 [100]	187 [100]	173 [100]
Age, years	52 (11)	50 (11)	51 (10)	51 (12)
Weight, kg	81 (14)	77 (13)	80 (14)	78 (13)
Height, cm	175 (9)	175 (10)	174 (9)	174 (10)
BMI, kg/m^2	26 (3)	25 (4)	26 (4)	26 (4)
BSA, m^2	1.96 (0.20)	1.91 (0.19)	1.94 (0.20)	1.93 (0.19)
SBP, mmHg	126 (13)	126 (13)	127 (14)	127 (14)
DBP, mmHg	76 (9)	76 (9)	76 (9)	77 (8)
Hypertension*, n [%]	106 [15]	29 [17]	54 [17]	37 [21]

Supplementary Table S4. Living kidney donor characteristics before donation in subgroups with and without five- and ten-year post-donation eGFR data available. (continued)

	Donated >5 year ago (N=856)		Donated >10 years ago (N=494)	
Baseline parameters	*5 year follow-up available*	*5 year follow-up not available*	*10 year follow-up available*	*10 year follow-up not available*
Use of antihypertensives	101 [15]	15 [9]	48 [15]	17 [10]
ACE inhibitors, n [%]	43 [6]	4 [2]	28 [9]	7 [4]
ARBs, n [%]	23 [3]	2 [1]	6 [2]	5 [3]
Betablockers, n [%]	39 [6]	7 [4]	22 [7]	5 [3]
Calcium antagonists, n [%]	16 [2]	5 [3]	7 [2]	5 [3]
Diuretics, n [%]	30 [4]	2 [1]	12 [4]	5 [4]
Statins, n [%]	22 [3]	3 [2]	7 [2]	4 [2]
Smoking, n [%]	157 [23]	54 [32]	78 [24]	54 [31]
eGFR, mL/min/1.73m^2	91 (13)	94 (14)	92 (13)	93 (14)
mGFR, mL/min	115 (22)	114 (20)	116 (22)	115 (21)
Δsk-eGFR, mL/min/1.73m^2	13 (8)	16 (11)	14 (8)	16 (10)
Δsk-eGFR%, %	14 (8)	17 (12)	16 (9)	17 (10)
Δsk-mGFR, mL/min	15 (8)	15 (8)	16 (8)	15 (8)
Serum creatinine, μmol/L	75 (13)	73 (12)	74 (12)	74 (12)
Serum glucose, mmol/L	5.3 (0.6)	5.2 (0.6)	5.2 (0.5)	5.2 (0.6)
HbA1c, %	5.5 (0.4)	5.4 (0.4)	5.5 (0.4)	5.5 (0.4)
Serum cholesterol, mmol/L	5.4 (1.0)	5.1 (1.0)	5.4 (1.0)	5.4 (1.0)
LDL	3.5 (0.9)	3.3 (0.8)	3.6 (0.8)	3.1 (0.4)
HDL	1.6 (0.5)	1.5 (0.6)	1.3 (0.3)	1.7 (1.2)
Triglycerides	1.4 (0.9)	1.3 (0.9)	1.5 (0.8)	1.4 (0.8)
Serum urea, mmol/L	5.5 (1.3)	5.3 (1.4)	5.5 (1.3)	5.4 (1.4)
Serum potassium, mmol/L	3.9 (0.3)	4.0 (0.4)	4.0 (0.4)	4.0 (0.4)
Serum sodium, mmol/L	141 (3)	141 (3)	141 (3)	141 (3)
Sodium excretion, mmol/24h	196 (73)	210 (82)	199 (74)	200 (67)
Protein/creatinine ratio, mg/mmol	0.0 [0.0 – 12.7]	7.9 [0.0 – 13.7]	0.0 [0.0 – 13.7]	0.0 [0.0 – 13.7]

Data presented as mean (standard deviation), median [interquartile range] or n [%].
*SBP >140 mmHg and/or DBP >90 mmHg
BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; ARBs: angiotensin receptor blockers; eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate; Δsk-eGFR: post-donation increase in single-kidney eGFR; Δsk-eGFR%: relative post-donation increase in single-kidney GFR; Δsk-mGFR: post-donation increase in single-kidney measured GFR; LDL: low-density lipoprotein; high-density lipoprotein.
Supplementary Table S4. Living kidney donor characteristics before donation in subgroups with and without five- and ten-year post-donation eGFR data available

Supplementary Table S5. Univariable associations of post-donation increase in single-kidney GFR and pre-donation parameters with eGFR at 5- and 10-years after donation.

	eGFR, 5 years after donation (n=693)		eGFR, 10 years after donation (n=321)	
	St. β	P	St. β	P
Δsk-GFR	0.56	<0.001	0.51	<0.001
eGFR	0.75	<0.001	0.67	<0.001
Age	-0.52	<0.001	-0.46	<0.001
HbA1C	-0.15	<0.001	0.004	0.95
SBP	-0.14	<0.001	-0.12	0.03
Sodium excretion	0.09	0.04	0.02	0.73
BMI	-0.08	0.03	-0.18	0.001
Female sex	-0.04	0.25	0.02	0.79
BSA	0.01	0.84	-0.04	0.46

Abbreviations: BMI: body mass index; BSA: body surface area; CI: confidence interval; Δsk-GFR: post-donation increase in single-kidney GFR; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; St. β: standardized beta.

Supplementary Table S6. Longitudinal association of the Δsk-GFR with protein-creatinine ratio (PCR) >15 mg/mmol at five and ten years post-donation.

	PCR, 5 years after donation (N=650)					
	Univariable			Multivariable		
	Exp(B)	P	95%CI	Exp(B)	P	95%CI
Female sex	1.73	0.02	1.11 – 2.69	1.62	0.03	1.04 – 2.54
BMI	0.93	0.04	0.87 – 1.00	0.94	0.08	0.89 – 1.01
eGFR	1.01	0.15	1.00 – 1.03	-	-	-
Δsk-GFR	1.02	0.21	0.99 – 1.04	1.01	0.36	0.99 – 1.04
SBP	1.01	0.39	0.99 – 1.02	-	-	-
Age	1.01	0.58	0.99 – 1.03	-	-	-
	PCR, 10 years after donation (N=301)					
	Univariable			Multivariable		
	Exp(B)	P	95%CI	Exp(B)	P	95%CI
eGFR	1.01	0.31	0.67 – 2.15	-	-	-
BMI	1.04	0.34	0.96 – 1.12	-	-	-
Age	1.02	0.34	0.98 – 1.05	-	-	-
SBP	1.01	0.40	0.99 – 10.3	-	-	-
Δsk-GFR	1.01	0.49	0.98 – 1.05	-	-	-
Female sex	1.20	0.54	0.67 – 2.15	-	-	-

Abbreviations: Δsk-GFR: post-donation increase in single-kidney GFR; PCR: protein/creatinine ratio; CI: confidence interval; BMI: body mass index; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure.

Supplementary Table S7. Multivariable associations of post-donation increase in single-kidney estimated glomerular filtration rate (eGFR), based on remaining vs total kidney cortical volume (Δsk-GFRKV), with eGFR at 5-years and 10-years post-donation.

	St. β	P	R^2
5-year eGFR (N=423)			
eGFR	0.60	<0.001	
Age	-0.12	<0.001	0.68
$\Delta sk\text{-}GFR_{KV}$	0.30	<0.001	
10-year eGFR (N=129)			
eGFR	0.62	<0.001	0.54
$\Delta sk\text{-}GFR_{KV}$	0.28	<0.001	

Abbreviations: eGFR: estimated glomerular filtration rate; Δsk-GFRKV: post-donation increase in single-kidney GFR based on cortical volume, corrected for kidney volume; St. β: standardized beta.

Supplementary Table S8. Multivariable associations of post-donation increase in single-kidney mGFR with mGFR at 5- and 10-years post-donation.

	St. β	P	R^2	R^2 change
5-year mGFR (N=529)				
Model 1				
mGFR	0.58	<0.001		
Age	-0.27	<0.001	0.73	
BSA	0.17	<0.001		
Model 2				
mGFR	0.61	<0.001		
Age	-0.18	<0.001	0.78	<0.001
BSA	0.14	<0.001		
Δsk-mGFR	0.25	<0.001		
10- year mGFR (N=236)				
Model 1				
mGFR	0.56	<0.001	0.61	
Age	-0.35	<0.001		
Model 2				
mGFR	0.57	<0.001		
Age	-0.28	<0.001	0.66	<0.001
Δsk-mGFR	0.25	<0.001		

Age, mGFR and BSA measured at pre-donation screening.
Abbreviations: BSA: body surface area; mGFR: measured glomerular filtration rate; Δsk-mGFR: post-donation increase in single-kidney mGFR; St. β: standardized beta

Supplementary Table S9. Characteristics of the validation cohort.

	Pre-donation	Post-donation	
		3 months	*Five years*
Number, n	647	647	647
Female sex, n [%]	372 [58]	372 [58]	372 [58]
White race, n [%]	628 [97]	628 [97]	628 [97]
Age, years	52 (13)	53 (13)	57 (13)
Weight, kg	79 (14)	79 (14)	81 (15)
Height, cm	172 (10)	172 (10)	172 (10)
BMI, kg/m^2	27 (4)	27 (4)	27 (4)
BSA, m^2	1.91 (0.20)	1.92 (0.20)	1.93 (0.20)
SBP, mmHg	129 (16)	130 (15)	132 (16)
DBP, mmHg	78 (9)	79 (8)	79 (8)
eGFR, mL/min/1.73m^2	93 (15)	58 (13)	61 (14)
Δsk-GFR, mL/min/1.73m^2	n.a.	12 (9)	n.a.

Data presented as mean (standard deviation) or n [%].
Abbreviations: BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; Δsk-GFR: post-donation increase in single-kidney GFR.

Supplementary Table S10. Multivariable linear regression model of pre-donation variables with the increase in single-kidney estimated glomerular filtration rate (eGFR) in the discovery and validation cohort.

	Discovery cohort		Validation cohort	
Univariable	**St. β**	**P**	**St.β**	**P**
eGFR	0.34	0.03	0.36	<0.001
Age	-0.33	<0.001	-0.33	<0.001
BMI	-0.11	0.001	-0.08	0.04
SBP	-0.10	0.001	-0.17	<0.001
BSA	-0.09	0.003	-0.08	0.04
Female sex	0.05	0.15	-0.01	0.88
Multivariable	**St. β**	**P**	**St. β**	**P**
eGFR	0.22	<0.001	0.23	<0.001
Age	-0.23	<0.001	-0.19	<0.001

Supplementary Table S10. Multivariable linear regression model of pre-donation variables with the increase in single-kidney estimated glomerular filtration rate (eGFR) in the discovery and validation cohort. (continued)

	Discovery cohort		Validation cohort	
Univariable	**St. β**	**P**	**St.β**	**P**
BSA	-0.13	<0.001	-0.09	0.01
BMI	-	-	-	-
Female sex	-	-	-	-
SBP	-	-	-	-

Multivariable model R2 main cohort= 0.16
Multivariable model R2 validation cohort= 0.15
Abbreviations: St. β: standardized beta; eGFR: estimated glomerular filtration rate; BMI: body mass index; SBP: systolic blood pressure; BSA: body surface area.

Supplementary Table S11. Associations of the relative Δsk-GFR (%) with 5- and 10-year eGFR.

	St. β	**P**	**R^2**
5-year eGFR (N=693)			
eGFR	0.67	<0.001	
Age	-0.10	<0.001	0.67
Δsk-GFR(%)	0.31	<0.001	
10-year eGFR (N=321)			
eGFR	0.66	<0.001	0.55
Δsk-GFR(%)	0.32	<0.001	

Abbreviations: Δsk-GFR(%): relative post-donation increase in single-kidney GFR, corrected for kidney volume; eGFR: estimated glomerular filtration rate; St. β: standardized beta.

9

CHAPTER

Early compensatory increase in single kidney eGFR after unilateral nephrectomy is associated with a lower long-term risk of eGFR decline

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ABSTRACT

Background

A stronger increase in single-kidney GFR (ΔskGFR) after living kidney donation has been associated with better long-term kidney function. Whether this also applies to non-donors is unknown. We evaluated whether ΔskGFR is associated with long-term risk of eGFR decline in individuals undergoing unilateral nephrectomy.

Methods

We included 1777 participants from the SCREAM cohort who underwent radical unilateral nephrectomy in Stockholm during 2006-2021. The ΔskGFR was calculated as the early (1-6 months) post-nephrectomy eGFR minus 50% of the pre-nephrectomy eGFR. The association between Δsk-GFR and the subsequent risk of progressive eGFR decline, defined as composite of an eGFR decline >30% compared to the early (6 months) post-nephrectomy eGFR or initiation of kidney replacement therapy, was analyzed using multivariable Cox regression.

Results

Mean age at nephrectomy was 68±11 years, 40% were female, 92% had kidney cancer, and mean pre-nephrectomy eGFR was 75±19 mL/min/1.73m^2. Median (IQR) Δsk-GFR was 11 (7-20) mL/min/1.73m^2. Pre-nephrectomy determinants of Δsk-GFR were age (St.β =-0.20, P<0.001) and pre-nephrectomy eGFR (St. β=0.14, P<0.001). During a median follow-up of 5 years (range 1-15 years), 178 participants developed progressive eGFR decline. Individuals with a Δsk-GFR above the median had a 42% lower risk of progressive eGFR decline (adjusted HR: 0.58, 95% CI: 0.42-0.80), compared to those with a lower Δsk-GFR, independent of baseline eGFR and age.

Conclusions

A stronger increase in single-kidney eGFR early after unilateral nephrectomy was associated with a lower long-term risk of progressive eGFR decline. Evaluation of Δsk-GFR could help identify patients at higher risk of progressive kidney function decline following unilateral nephrectomy.

INTRODUCTION

Physical resilience is defined as "the ability to resist functional decline or recover functional health following a stressor" and is (partly) determined by reserve capacity, defined as "the potential capacity of a cell, tissue or organ system to function beyond its basal level in response to alterations in physiologic demands".[1] In the human kidney, resilience is demonstrated by maintenance of glomerular filtration rate (GFR) during early kidney damage or by recovery of GFR after an acute reduction of nephrons, for example unilateral nephrectomy.[2,3] Since no new nephrons are formed during life, this is achieved by increasing the single-nephron GFR in the remaining nephrons.[2]

An increase in single-nephron GFR may have deleterious effect as hyperfiltration may lead to podocyte detachment, proteinuria, nephrosclerosis, further nephron loss and subsequent adverse outcomes.[4–8] On the other hand, it has been suggested that the post-nephrectomy increase in single-kidney GFR is probably driven by an increased renal plasma flow and filtration fraction instead of an increased glomerular hydraulic pressure.[9,10] In line with the latter hypothesis, we recently demonstrated that a higher post-donation increase in single-kidney GFR in living kidney donors is an independent predictor of better long-term kidney function.[11] Whether this principle only applies to the highly selected kidney donor population or may be extended to other populations is unknown.

In this study, we evaluate the prognostic value of the short-term increase in single-kidney GFR in a population of patients undergoing unilateral nephrectomy for other reasons than kidney donation, mostly malignancy. We hypothesized that a higher post-nephrectomy increase in single-kidney GFR reflects a higher reserve capacity in the remaining kidney, and that this would be associated with a lower risk of subsequent kidney function decline.

METHODS

Data Source

We used data from the Stockholm Creatinine Measurements (SCREAM) project. SCREAM is a healthcare utilization cohort from the region of Stockholm, Sweden, covering the period from 2006 to 2021.[12] A single healthcare provider in the Stockholm region provides universal and tax-funded healthcare to 20% to 25% of the population of Sweden. Using unique personal identification numbers, SCREAM linked regional and national administrative databases that hold

complete information on demographics, healthcare utilization, laboratory tests undertaken, dispensed drugs, diagnoses, and vital status. The Regional Ethical Review Board in Stockholm approved the study (reference 2017/793-31); informed consent was not deemed necessary because all data were de-identified at the Swedish Board of Health and Welfare.

Study population and study design

A flowchart of the study population selection for this study is shown in **Figure S1**, and a schematic overview of the study design is shown in **Figure 1**. Inclusion criteria were adults (>18 years) undergoing radical unilateral nephrectomy (Nomesco procedure codes: KAC00, KAC01, KAC20 and KAC21[13], **Table S1**), with at least one creatinine measurement within one year before nephrectomy, at least one during the exposure period (four weeks to six months post-nephrectomy), and at least two creatinine measurements during follow-up (after six months post-nephrectomy) (**Figure 1**). Exclusion criteria were having a diagnosis of living kidney donor (ICD10-SE: Z52.4) or history of kidney replacement therapy (KRT, ascertained by linkage with the Swedish Renal Registry). Additionally, we excluded patients with a diagnosis of urinary flow obstruction (ICD10-SE: N13) or urolithiasis (ICD10-SE: N20-N23), because patients that undergo unilateral nephrectomy for these reasons are likely to compensate kidney function of the contralateral healthy kidney prior to nephrectomy.[14]

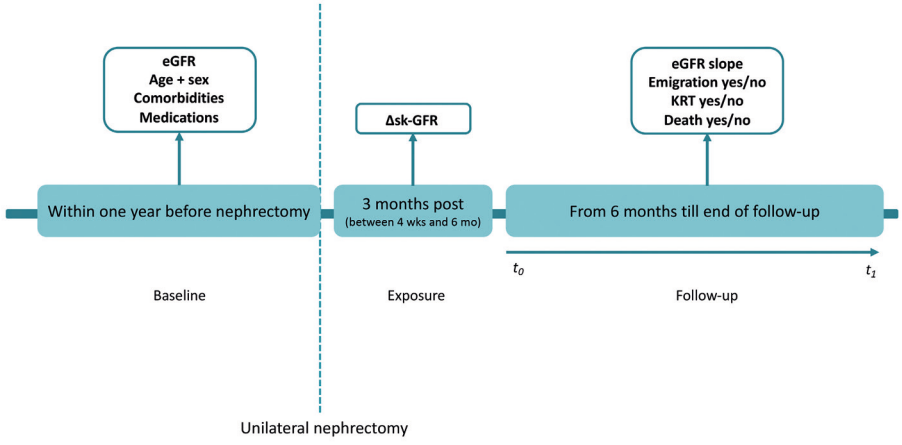

Figure 1. Schematic overview of study period and data collection at each time point.

Baseline characteristics were collected prior to nephrectomy, the exposure (Δsk-GFR) was calculated as short-term post-nephrectomy eGFR minus 50% of pre-nephrectomy eGFR. Follow-up period started after exposure period at six months.

Exposure, covariates and outcomes

For study participants, we extracted all serum and plasma creatinine measurements performed in connection to healthcare encounters, and used them to estimate glomerular filtration rate (eGFR) using the 2009 CKD-EPI equation without the race coefficient.[15] Our exposure was the short-term post-nephrectomy increase in single-kidney GFR (Δsk-GFR), calculated as the eGFR between 1 and 6 months after nephrectomy minus 50% of the pre-nephrectomy eGFR.[11] The pre-nephrectomy eGFR value was defined as the median eGFR of all measurements in the year prior to the date of nephrectomy. The post-nephrectomy eGFR value was defined as the median eGFR of all measurements during the one to six months after nephrectomy (**Figure 1**). The baseline for post-nephrectomy follow-up was set at month 6 after the nephrectomy date, and on this date all other study covariates were derived, and follow-up began.

Study covariates included age, sex, pre-nephrectomy eGFR, comorbidities and ongoing medications (see **Table S1** for detailed definitions and look back periods for ascertainment).

The study outcome was progressive eGFR decline, defined as a composite endpoint of a decline in eGFR greater than 30% compared to baseline (6 months post-nephrectomy) eGFR, or initiation of KRT (i.e. dialysis or kidney transplantation). To reduce outcome misclassification bias owing to intrinsic eGFR variability, and to confirm whether eGFR declines were sustained over time, we used a linear interpolation method[16]. In brief, for each study participant we fitted a linear regression line through all outpatient eGFR measurements after index date. To be considered a sustained eGFR decline of more than 30% relative to post-nephrectomy eGFR, the linear regression slope needed to be negative, and the threshold of a 30% difference needed to be crossed before the last available measurement. The time-to-event outcome was then defined as the interpolated moment at which the linear regression line reached an eGFR 30% lower. Patients were followed until event, death, migration or end of follow up (31st Dec 2021), whichever occurred first. Date of death was retrieved from the Swedish cause of death register.

Statistical analyses

Data were presented as mean with standard deviation or as median with interquartile range when appropriate for continuous variables and as number with percentage for categorical variables. In univariable linear regression analyses, we investigated whether age, sex, pre-nephrectomy eGFR, hypertension, dia-

betes or cardiovascular disease were associated with the Δsk-GFR. Variables associated with Δsk-GFR with a P-value <0.2 were added to a multivariable linear regression model. Next, we graphically depicted the cumulative incidence of our outcome progressive eGFR decline over time for patients with low Δsk-GFR (above the median) vs. patients with high Δsk-GFR (below or equal to the median) using Kaplan-Meier plots. Using multivariable Cox proportional hazard's regression, we investigated the association between a high Δsk-GFR (defined by an increase above the median value) and the risk of developing progressive eGFR decline. Identified confounders were age, sex and pre-nephrectomy eGFR, and were adjusted for in the multivariable model.

We explored potential effect modification by multiplicative interaction terms across subgroups of age, sex, hypertension-, diabetes- and cardiovascular disease status. Sensitivity analyses evaluated the robustness of our results by exploring alternative thresholds of Δsk-GFR (highest and lowest quartile instead of the median), and considering death as a competing-risk events through Fine and Gray models. Analyses were performed with R Software (RStudio version 2022.07.2-576 "Spotted Wakerobin", R version 4.2.1).

RESULTS

Population characteristics

A total of 1777 adults undergoing radical unilateral nephrectomy met the inclusion and exclusion criteria (see flow chart in **Figure S1**). The median Δsk-GFR was 11 (interquartile range 7 to 20) mL/min/1.73m^2. Patients were subsequently divided into categories according to low (≤11 mL/min/1.73m^2) or high (>11 mL/min/1.73m^2) Δsk-GFR value. Patient characteristics are shown in **Table 1**. Mean age of the study population was 68±11 years, pre-nephrectomy eGFR was 75±19 mL/min/1.73m^2, and 703 (40%) were female. Mean exposure eGFR (i.e. eGFR between 1 and 6 months post-nephrectomy), was 51±17 mL/min/1.73m^2. The majority (92%) of patients had a diagnosis of kidney cancer in three years prior to nephrectomy, and 20% had diabetes. As many as 62% had a clinical diagnosis of hypertension. Patients in the high Δsk-GFR group were younger and had a higher pre-nephrectomy eGFR than patients in the low Δsk-GFR group.

Table 1. Baseline characteristics of the study population.

	Overall	Low Δsk-GFR ≤11 mL/min/1.73m²	High Δsk-GFR >11 mL/min/1.73m²
N (%)	1777 (100%)	865 (49%)	912 (51%)
Age, years	67.9 [11.3]	70.8 [9.9]	65.0 [12.0]
Age categories			
19-40 years	32 (1.8%)	5 (0.6%)	27 (3.0%)
41-65 years	610 (35%)	212 (25%)	412 (45%)
65+ years	1,106 (63%)	646 (75%)	473 (51%)
Female sex, N (%)	703 (40%)	343 (40%)	368 (40%)
Pre-nephrectomy eGFR, mL/min/1.73m²	74.9 [19.3]	69.7 [19.3]	78.3 [20.7]
Pre-nephrectomy eGFR categories			
>=60 mL/min/1.73m²	1,364 (78%)	623 (72%)	747 (82%)
30-59 mL/min/1.73m²	361 (21%)	212 (25%)	149 (16%)
<30 mL/min/1.73m²	23 (1.3%)	28 (3.2%)	16 (1.8%)
Exposure eGFR, mL/min/1.73m²	50.6 [17.0]	39.8 [11.7]	60.8 [14.7]
Comorbidities, N (%)			
Hypertension	1,091 (62%)	606 (70%)	511 (56%)
Diabetes	344 (20%)	198 (23%)	151 (17%)
Obesity	167 (9.6%)	83 (9.6%)	86 (9.4%)
Cardiovascular disease	433 (25%)	260 (30%)	183 (20%)
Cancer*	1,654 (95%)	806 (93%)	862 (95%)
Renal cancer*	1,615 (92%)	807 (93%)	819 (90%)
Kidney trauma	7 (0.4%)	2 (0.2%)	6 (0.7%)
Ongoing medications			
Antihypertensives	1,684 (96%)	843 (97%)	869 (95%)
Antidiabetics	256 (15%)	144 (17%)	116 (13%)

*In three years prior to nephrectomy
Data presented as N (%) for binary variables and mean [standard deviation] for continuous variables. For diagnosis and ATC codes used to extract comorbidities and medications, see Table S1.
Abbreviations: Δsk-GFR = delta single kidney estimated glomerular filtration rate (calculated as short-term post-nephrectomy eGFR – 50% pre-nephrectomy eGFR); eGFR = estimated glomerular filtration rate.

Pre-nephrectomy determinants of the Δsk-GFR

In univariable analyses, age (St.β=-0.30, P<0.001), pre-nephrectomy eGFR (St.β=0.27, P<0.001), a diagnosis of hypertension (St.β=-0.16, P<0.001), diabetes

(St.β=-0.08, P=0.001) and cardiovascular disease (St.β=-0.13, P<0.001) were significantly associated with the Δsk-GFR (**Table 2**). When including these variables in a multivariable model, only age (St.β=-0.20, P<0.001) and pre-nephrectomy eGFR (St.β=0.14, P<0.001) remained as independent determinants of the Δsk-GFR (model R^2=0.11).

Δsk-GFR and outcomes

During a median follow-up 4.8 (range 2.3 – 8.3 years), 178 patients developed progressive eGFR decline and 543 patients died before experiencing progressive eGFR decline. **Figure 2** depicts the Kaplan-Meier cumulative incidence of progressive eGFR decline events, which was higher for patients in the low Δsk-GFR compared vs. patients in the high Δsk-GFR group (P-value log-rank test <0.001). Compared to patients in the low Δsk-GFR group, those in the high Δsk-GFR group had a 42% lower risk of progressive eGFR decline, independent of age, sex, pre-nephrectomy eGFR (hazard ratio (HR): 0.58, 95% CI: 0.42 – 0.80, **Table 3**). Results were similar after accounting for death as a competing risk (subHR: 0.67, 95% CI: 0.48 – 0.94).

Table 2. Univariable and multivariable linear regression analysis of pre-nephrectomy predictors of Δsk-GFR.

	Univariable		Multivariable	
	St.β	**P**	**St.β**	**P**
Age, years	-0.30	<0.001	-0.20	<0.001
Sex	-0.03	0.28	-	-
Pre-nephrectomy eGFR, mL/min/1.73 m^2	0.27	<0.001	0.14	<0.001
Hypertension	-0.16	<0.001	-0.04	0.08
Diabetes	-0.08	0.001	-0.04	0.12
Cardiovascular disease	-0.13	<0.001	-0.03	0.18

All variables with P-value <0.2 were included in the multivariable model.
Multivariable model R^2=0.11
Abbreviations: Δsk-GFR = delta single kidney estimated glomerular filtration rate (calculated as short-term post-nephrectomy eGFR – 50% pre-nephrectomy eGFR); eGFR = estimated glomerular filtration rate.

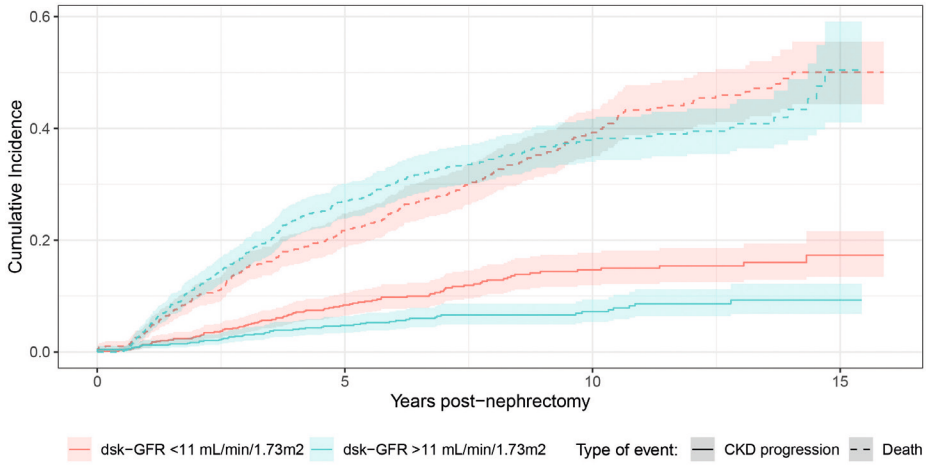

Figure 2. Kaplan-Meier plot showing cumulative incidence of progressive eGFR decline and death for low Δsk-GFR vs. high Δsk-GFR.

The Δsk-GFR was dichotomized based on the median value (11 mL/min/1.73m^2): low= Δsk-GFR ≤11 mL/min/1.73m^2; high= Δsk-GFR >11 mL/min/1.73m^2. Progressive eGFR decline was a composite endpoint of a decline in eGFR greater than 30% compared to baseline (6 months post-nephrectomy) eGFR, or initiation of KRT (i.e. dialysis or kidney transplantation). Abbreviations: Δsk-GFR = delta single kidney estimated glomerular filtration rate (calculated as short-term post-nephrectomy eGFR – 50% pre-nephrectomy eGFR); eGFR = estimated glomerular filtration rate.

Subgroup analyses

The association of the Δsk-GFR with progressive eGFR decline did not differ across strata of age, sex, hypertension, diabetes or cardiovascular disease (**Table 4**, P value for interaction 0.95, 0.37, 0.87, 0.90 and0.57 respectively). When the Δsk-GFR was dichotomized based on the highest quartile (≥19 mL/min/1.73m^2) vs. the rest of the cohort (<19 mL/min/1.73m^2), a higher Δsk-GFR was not significantly associated with a lower risk of progressive eGFR decline (adjusted HR: 0.87, 95% CI: 0.60 to 1.28, **Table S3**). There were 38 events in the highest Δsk-GFR quartile vs. 140 events in the rest of the cohort (total 178 events). A Δsk-GFR above the lowest quartile (≥7 mL/min/1.73m^2) was significantly associated with a 54% lower risk of progressive eGFR decline (adjusted HR: 0.46, 95% CI: 0.34 to 0.62, **Table S2**). There were 82 events in the lowest Δsk-GFR quartile vs. 96 events in the rest of the cohort (total 178 events).

Table 3. Association between Δsk-GFR categories with the risk of progressive eGFR decline.

	Number of events/ patients	IR (95% CI) per 1000 person years	10-year absolute risk (%, 95% CI)	HR (95% CI)*	Subdistribution HR (95% CI)**
Low Δsk-GFR	117/865	24 (20 - 29)	15% (12% -18%)	1.00 (ref)	1.00 (ref)
High Δsk-GFR	61/912	12 (9 - 15)	7% (5% - 9%)	0.58 (0.42 - 0.80)	0.67(0.48 - 0.94)

*Risk of progressive eGFR decline with Cox regression, censoring for death and emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.
**Risk of progressive eGFR decline with Fine and Gray models considering death as a competing event, and censoring for emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.
The Δsk-GFR was dichotomized based on the median value (11 mL/min/1.73m^2): low= Δsk-GFR ≤11 mL/min/1.73m^2; high= Δsk-GFR >11 mL/min/1.73m^2.
Abbreviations: Δsk-GFR = delta singke-kidney glomerular filtration rate; CKD = chronic kidney disease; CI = confidence interval; eGFR = estimated glomerular filtration rate; HR = hazard ratio; IR = incidence rate; KRT = kidney replacement therapy.

Table 4. Subgroup analyses by age, sex and absolute post-nephrectomy eGFR

	N Δsk-GFR ≤11 mL/min/1-73m^2	N Δsk-GFR >11 mL/min/1-73m^2	HR (95% CI), ref: Δsk-GFR ≤11 mL/min/1-73m^2	P-value for interaction
Sex				
Female	343	368	0.71 (0.41 to 1.23)	0.37
Male	522	544	0.52 (0.35 to 0.77	
Age				
≥70 years	355	551	0.58 (0.36 to 0.94)	0.95
<70 years	510	361	0.51 (0.33 to 0.79)	
Hypertension				
Yes	606	511	0.59 (0.41 to 0.84)	0.87
No	259	401	0.62 (0.32 to 1.22)	
Diabetes				
Yes	198	151	0.60 (0.34 to 1.13)	0.90
No	667	761	0.62 (0.41 to 0.87)	
Cardiovascular disease				
Yes	260	182	0.67 (0.39 to 1.16)	0.57
No	605	730	0.55 (0.37 to 0.81)	

Abbreviations: Δsk-GFR = delta singke-kidney glomerular filtration rate; CKD = chronic kidney disease; CI = confidence interval; eGFR = estimated glomerular filtration rate; HR = hazard ratio; N = number.

Sensitivity analysis
We performed a sensitivity analysis in which we ran the main analysis (cox regression model with outcome progressive eGFR decline) only in patients with a history of kidney cancer (N=1626), which did not affect our results, as shown in **Table S4**.

DISCUSSION

The purpose of this study was to investigate the association between the short-term increase in single-kidney GFR (Δsk-GFR) after radical unilateral nephrectomy and the long-term risk of subsequent kidney function loss (>30% reduction in eGFR or kidney failure). The main result was that patients with a higher Δsk-GFR had a 42% lower risk of progressive eGFR decline, independent of age, sex and pre-nephrectomy eGFR. Individuals with a very limited increase in single-kidney eGFR, reflecting very little renal reserve, seemed to be particularly at risk of subsequent kidney function loss.

Whether an increase in single-kidney eGFR is linked with beneficial or adverse outcomes has been subject of discussion, and may be context-dependent. Animal studies have shown that hyperfiltration in remnant nephrons after subtotal nephrectomy can lead to glomerular damage, proteinuria, nephrosclerosis and subsequent progressive nephron loss in 5/6-nephrectomy rat models.[8,17,18] These findings were the foundation of the "Brenner hypothesis", stating that after substantial loss of nephrons, hyperfiltration in remnant nephrons, mediated by increased intraglomerular pressure, leads to a vicious circle of further nephron loss and progressive kidney function decline.[7] In keeping with this hypothesis, concerns have been raised about compensatory hyperfiltration in the remaining kidney after unilateral nephrectomy. However, other (potentially less harmful) mechanisms may be involved in compensatory hyperfiltration as well, such as suppression of growth inhibitory genes.[19,20] Favorable outcomes after unilateral nephrectomy in healthy kidney donors support this.[9,21] In line, results of the current study show that a stronger increase in single kidney GFR after unilateral nephrectomy, indicating more hyperfiltration, is associated with progressive eGFR decline. Possibly, there is a range of nephron loss that the kidney can tolerate without inducing pathophysiological pathways leading to further nephron loss. The extent of damage present in the remaining nephrons may also be of importance, and therefore a comparison of the current study population with the healthy donor population in our previous work is of interest.

The results of this study align with previous findings about the post-donation increase in single-kidney GFR in living kidney donors[11], despite differences in patient populations. Patients in the current study had lower pre-nephrectomy eGFR, were older, had more comorbidities and the majority was diagnosed with kidney cancer and may have received chemotherapy prior to nephrectomy. It has been shown that in >60% of patients undergoing unilateral nephrectomy for renal cell carcinoma, the renal parenchyma and vasculature show evident pathologic abnormalities.[22] Consequently, it could be hypothesized that these patients are more prone to hyperfiltration, accompanied by increased glomerular pressure, and resulting in glomerular damage and subsequent adverse outcomes. However even in this population, we found that a stronger increase in single-kidney GFR post-nephrectomy is independently associated with a reduced risk of progressive kidney function decline. While it is possible that some patients experienced malignant post-nephrectomy hyperfiltration leading to adverse outcomes[22], overall, more pronounced hyperfiltration was not an indicator of unfavorable outcomes. In line, a previous study showed that renal blood flow after unilateral nephrectomy in patients with renal cell carcinoma increased at one week and one month after nephrectomy and returned to pre-nephrectomy values at three months.[23] A more pronounced Δsk-GFR may reflect more reserve capacity of the kidney, while further studies are needed to identify potential differences between benign and malignant compensatory pathways.

The majority of patients in this study that underwent radical unilateral nephrectomy had a history of kidney cancer. This might raise concerns about pre-nephrectomy compensation of the contralateral healthy kidney for reduced single-kidney function of the affected kidney, which may affect applicability of our Δsk-GFR equation. Previous studies show that increased tumor size (>7 cm) negatively affects post-nephrectomy compensation of the contralateral remaining kidney, indicating that compensation may have (partly) occurred prior to nephrectomy.[24,25] Yet, Song et al. found that patients undergoing unilateral nephrectomy for kidney cancer had a volume ratio of contralateral healthy kidney compared to diseased kidney of 1.03 in a population with mean tumor size of 6 cm, suggesting equal kidney size.[26] In the group undergoing unilateral nephrectomy due to urolithiasis, strictures, pyelonephritis or tuberculosis in the same study, this ratio was 2.81, which supports our method of excluding patients with urolithiasis or urinary flow obstruction.[26] A limitation of our study is that we did not have information on tumor size or kidney volume a, which could influence compensation of the contralateral healthy kidney. However, the distribution of the Δsk-GFR in the current study was highly comparable to the Δsk-GFR study in living kidney donors[11],

which suggests that our equation of the Δsk-GFR is not (strongly) impacted by pre-nephrectomy compensation of the contralateral healthy kidney. Additionally, remnant kidney function is positively associated with the Δsk-GFR in most studies in kidney cancer patients, which contradicts pre-nephrectomy compensation of the remaining kidney.[27–29]

Other independent determinants of Δsk-GFR were age (inverse) and pre-nephrectomy eGFR (positive), in line with previous findings in both radical nephrectomy patients and kidney donors.[28,30–35] All previous studies including kidney volume of the remnant kidney found an independent and positive association with post-nephrectomy compensation of GFR in both populations.[11,29–31,34] Some studies also identified BMI, hypertension, sex and presence of cysts as determinants.[29–32,35] However, compensation was defined differently in all studies and the overall explained variance by the above-mentioned determinants was low. Our study underlines the relevance of identifying more predictors of the Δsk-GFR, in order to identify patients at risk of progressive kidney function decline.

Strengths of this study include the complete health care coverage from a region with universal tax-funded health care and the availability of pre-nephrectomy, exposure and follow-up measurements in 1777 patients. Moreover, linear interpolation of eGFR during follow-up minimizes the risk of falsely detecting 30% eGFR decline by a transient drop in eGFR. However, it should be acknowledged that due to the retrospective and observational design of the study, no pre-specified follow-up time points were available and possibly, patients with stronger kidney function decline were likely followed up more extensively. Yet, the incidence rates of creatinine testing in patients with high Δsk-GFR vs. low Δsk-GFR and rates were comparable. Another limitation is the absence of information on tumor size in the kidney cancer patients or information on pre-nephrectomy kidney size or split kidney function. However, as discussed more extensively above, the impact of these parameters might have been limited.

In conclusion, we found that a stronger Δsk-GFR after radical unilateral nephrectomy is independently associated with a lower risk of long-term progressive kidney function decline. A higher Δsk-GFR may therefore be an expression of resilience, possibly driven by the reserve capacity of the kidney. Future studies are needed to investigate post-nephrectomy adaptive mechanisms in both healthy individuals and patients, thereby improving understanding of the reserve capacity of the kidney. Such studies may provide important insight in kidney physiology, and shape protocols to provide better follow-up and care after unilateral nephrectomy.

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SUPPLEMENTARY MATERIAL

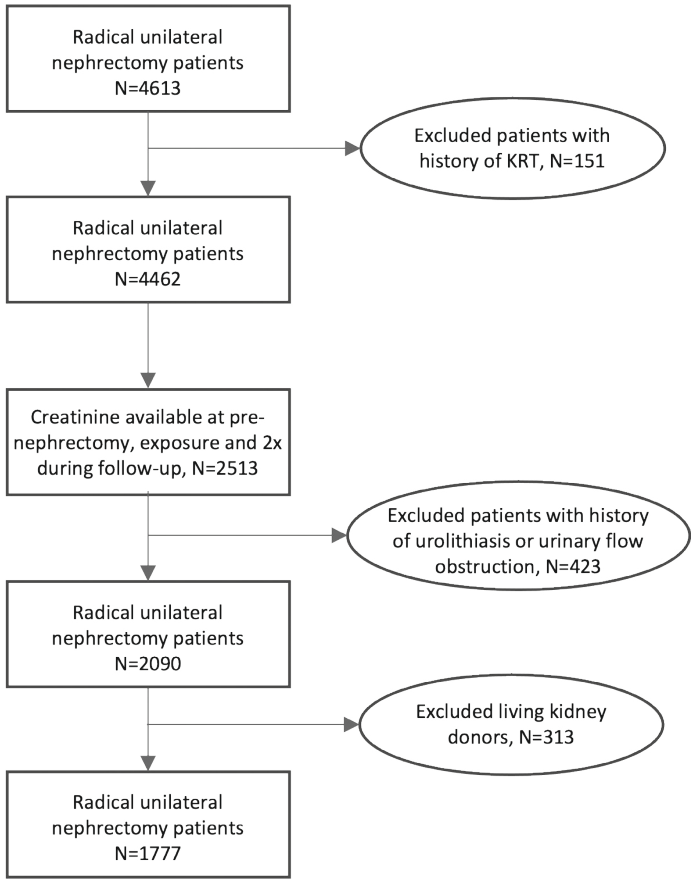

Figure S1. Flowchart of study population selection. KRT = kidney replacement therapy.

Table S1. Diagnosis codes and ATC codes used to extract comorbidities and medications.

	Code	Look back period
Procedures	*Nomesco procedure codes*	
Radical unilateral nephrectomy	KAC00, KAC01, KAC20 and KAC21	Any time during study period
Comorbidities	*ICD-10-SE codes*	
Living kidney donor	Z52.4	Any time pre-nephrectomy
Hypertension	I10-I15	Any time pre-nephrectomy
Diabetes	E10-E14	Any time pre-nephrectomy
Obesity	E65 and E66	Any time pre-nephrectomy
Cardiovascular disease	I20-I25, I110, I130, I132, I134-I137, I142-I150,	Any time pre-nephrectomy
Cancer*	C0-C3, C41-C49, C5-C9, D01-D09	Three years pre-nephrectomy
Renal cancer*	C64, C65, C790, C091, D300, D301, D410, D411	Three years pre-nephrectomy
Kidney trauma	S37	Any time pre-nephrectomy
Urolithiasis	N20-N23	Any time pre-nephrectomy
Unirnary flow obstruction	N13	Any time pre-nephrectomy
Medications	*ATC codes*	
Antihypertensives	C02, C03, C07-C09	Any time pre-nephrectomy
Antidiabetics	A10	Any time pre-nephrectomy

Table S2. Association between Δsk-GFR categories and the risk of progressive eGFR decline.

	Number of events/ participants	IR (95% CI) per 1000 person years	10-year absolute risk (%, 95% CI)	HR (95% CI)*	Subdistribution HR (95% CI)**
Highest quartile vs. rest cohort					
Low Δsk-GFR	140/1289	19 (16 to 22)	12% (10% to 14%)	1.00 (ref)	1.00 (ref)
Highest quartile Δsk-GFR	38/488	14 (10 to 20)	9% (6% to 12%)	0.87 (0.60 to 1.28)	0.94 (0.63 to 1.38)
Lowest quartile vs. rest cohort					
Lowest quartile Δsk-GFR	82/436	36 (28 to 44)	18% (14% to 23%)	1.00 (ref)	1.00 (ref)
High Δsk-GFR	96/1341	12 (10 to 15)	9% (7% to 11%)	0.46 (0.34 to 0.62)	0.61 (0.43 to 0.84)

*Risk of progressive eGFR decline with Cox regression, censoring for death and emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.
**Risk of progressive eGFR decline with Fine and Gray models considering death as a competing event, and censoring for emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.
For both models, the Δsk-GFR was dichotomized based on the highest or lowest quartile vs. the rest of the cohort
Highest quartile Δsk-GFR: (≥19 mL/min/1.73m^2)
Lowest quartile Δsk-GFR: (<7 mL/min/1.73m^2)
Abbreviations: Δsk-GFR = delta singke-kidney glomerular filtration rate; CKD = chronic kidney disease; CI = confidence interval; eGFR = estimated glomerular filtration rate; HR = hazard ratio; IR = incidence rate; KRT = kidney replacement therapy.

Table S3. Association between Δsk-GFR categories and the risk of progressive eGFR decline in only patients with kidney cancer

	Number of events/ participants	IR (95% CI) per 1000 person years	10-year absolute risk (%, 95% CI)	HR (95% CI)*	Subdistribution HR (95% CI)**
Low Δsk-GFR	101/807	21 (17 to 26)	15% (12% to 18%)	1.00 (ref)	1.00 (ref)
High Δsk-GFR	47/819	10 (7 to 13)	7% (5% to 9%)	0.60 (0.42 to 0.86)	0.65 (0.45 to 0.92)

*Risk death with Cox regression, censoring for emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.

**Risk of progressive eGFR decline with Fine and Gray models considering death as a competing event, and censoring for emigration. Model adjusted for pre-nephrectomy eGFR, age and sex.

The Δsk-GFR was dichotomized based on the median value (11 mL/min/1.73m^2): low= Δsk-GFR ≤11 mL/min/1.73m^2; high= Δsk-GFR >11 mL/min/1.73m^2.

Abbreviations: Δsk-GFR = delta singke-kidney glomerular filtration rate; CI = confidence interval; eGFR = estimated glomerular filtration rate; HR = hazard ratio; IR = incidence rate; KRT = kidney replacement therapy.

10

CHAPTER

Discussion

In 2021 in the Netherlands, 102 patients with kidney failure died while waiting for a kidney transplant, and 142 patients on the waiting list became too ill to undergo kidney transplantation.[1] Every year, about 1400 new patients enter the kidney transplant waiting list, while the number of kidney transplantations performed every year is between 950 and 1000.[1] This has led to an increase in the waiting list from 650 patients in 2017 to 877 patients in 2021.[1] Despite exciting news about increased transplantation rates in 2022 due to a new donor law in the Netherlands[2], the waiting list is expected to increase due to rising incidence of chronic kidney disease (CKD).[3,4] Currently, 50% of the annually performed kidney transplantations in the Netherlands are with kidneys from living donors.[1] Living kidney donors are carefully selected based on kidney function and general health. Generally, outcomes for living kidney donors are excellent: although risks of developing kidney failure are increased when compared to healthy controls, absolute risks are still low and even lower than risks of the general population.[5] For recipients, outcomes are mostly better when they receive a kidney from a living donor compared to a deceased donor kidney.[6,7] It is therefore needless to say that living kidney donors play an extremely important role in treating kidney failure. Due to the pressure on the waiting list and the favorable outcomes of living kidney donors, selection criteria for living kidney donors tend to liberalize.[8] Compared to 20 years ago, nowadays more donors with overweight or well-controlled hypertension are allowed to donate. It is of utmost importance to keep post-donation risks minimal, and therefore accurate prediction of post-donation kidney function and understanding to which extent a donor can compensate for the loss of one kidney without harming the remaining kidney is highly relevant.

One of the most important parts of living kidney donor evaluation is therefore to estimate whether the donor will retain sufficient kidney function after donation to maintain good health. This is done by assessment of kidney function as well as screening for risk factors that might affect kidney function in the future. To date, there is no consensus about how kidney function should be assessed in potential donors, which hampers risk prediction and potentially impairs selection efficiency. This is partly due to the various measured and estimated GFR assessment methods that are available which are affected by measurement error and/or limited availability, but also because predicting how much the remaining kidney will compensate after donation is troublesome. While 50% of the functioning kidney mass is removed, the remaining kidney exhibits a reserve force enabling it to increase post-donation single-kidney GFR up to 65-75% of the pre-donation value, but the extent of compensation varies between individuals for unknown reasons.[9,10] Also, it is not entirely known whether this compensatory response

is at the expense of remaining kidney health. Potential kidney donors consider post-donation kidney function the most important outcome to decide whether they want to take the risk of donating a kidney.[11] Thus, accurate prediction of post-donation kidney function will contribute to well informed shared decision making by the clinician and the donor.

Aims

In this thesis, we explored existing and novel methods to assess pre-donation and predict post-donation kidney function (**Part A**). Next, in **Part B**, we aimed to focus on kidney health on the microscopic level. We studied variations in microstructural parameters measured in kidney biopsies from kidney donors and investigated their relations with clinical characteristics and kidney function. Lastly, in **Part C**, we investigated the prognostic value of the post-donation compensatory increase in GFR and its pre-donation determinants. While studies in **Part C** were relevant for understanding GFR compensation and its relation with long-term outcomes in living kidney donors, they also served a broader context in which we generated hypotheses on the reserve capacity of the kidney that might be relevant for other fields than kidney donation as well.

PART A

Measured vs. estimated GFR in living kidney donor screening

Estimation of GFR by one of the existing eGFR formulas that are usually based on serum creatinine, is often used as preliminary screening before the full donor evaluation takes place, sometimes even in the primary care setting. As such, it is highly important that this estimate is accurate. Of note, most equations were developed in populations with reduced GFR resulting in inaccuracy (mostly underestimation) of GFR in higher GFR ranges.[12] It has been shown that relying on eGFR only could lead to improper acceptance or denial of potential donor candidates and some studies and centers therefore favor the use of measured GFR for donor selection (mGFR).[13] Measuring the clearance of inulin is considered the gold standard, but complexity, costs, limited availability and patient inconvenience of this method limit use in clinical practice.[14] Other exogenous markers such as iohexol, ^{51}Cr-EDTA and ^{125}I-iothalamate can be used as well, but they all have bias compared to inulin and are also costly and laborious.[15,16] These limitations hamper implementation of mGFR in routine clinical practice.[17] At the same time, we demonstrated in **Chapter 2** that at a group level, routine use of mGFR does not seem to result in acceptance of donors with lower eGFR, despite the known underestimation of mGFR by eGFR in living kidney donors.[18]

Moreover, we found no differences in five-year post-donation eGFR between centers that use mGFR-based donor screening and centers that use eGFR-based donor screening. This suggests that routine use of mGFR might offer no or only little benefits, especially in relation to the practical and financial limitations of this method. The benefit of mGFR for donor screening, if at all present, might therefore only apply to a subgroup of donor candidates.

In an attempt to identify determinants of discrepancy between eGFR and mGFR, we compared characteristics of donors with >10 and >20 mL/min/1.73m^2 difference between eGFR and mGFR to donors with differences between eGFR and mGFR below these thresholds in **Chapter 2**. Because serum creatinine is affected by muscle mass[19], we expected to find differences in potential surrogates for muscle mass in donors with a large bias between creatinine-based eGFR and mGFR, such as age, sex and body size measurements. However, none of these characteristics were different between donors with and without underestimation of mGFR by eGFR in **Chapter 2**.[18] It could be that determinants of muscle mass did not play a role in underestimation here, but it should also be noted that age, sex and body size measurements as weight, height, body mass index and body surface area might not be perfect surrogates for muscle mass. Additionally, influences of muscle mass on inaccuracy of eGFR might apply to donors in both the lower ranges and the higher ranges of muscle mass, which might balance out the values of these surrogates. In **Chapter 3**, we found that in donors with muscle mass (assessed by 24-hour creatinine excretion) in the highest and lowest quartile, plasma creatinine was less strongly associated with mGFR, resulting in weaker performance of the creatinine-based CKD-EPI equation in this group. In this study, we show that inaccuracy of creatinine-based eGFR due to effects of muscle mass is not only a problem in people with extremely low or high muscle mass (e.g. due to anorexia or body building), but in fact can be problematic in 50% of the donor population (highest and lowest quartile). Thus, issues with creatinine-based eGFR in potential living kidney donors are two-fold: 1) poor performance because most commonly used equations are derived from non-donor populations; and 2) estimates are possibly (strongly) impacted by variation in muscle mass.

Measurement of serum creatinine is widely implemented for kidney function assessment; it is cheap and easy and many clinicians are familiar with its limitations with regard to influences of muscle mass.[20] However, on top of the issues described above, intra-individual variation (4-6%) and inter-assay variation (2-5%) also account for differences in serum creatinine.[20] Combining multiple markers

of kidney function in an eGFR equation could diminish the impact of such non-GFR-driven influences. This has led to improvement of accuracy and precision of eGFR equations when they were updated to include both creatinine and cystatin C.[21,22] In **Chapter 3**, we showed that also in living kidney donors, accuracy and precision of the CKD-EPI equation increases when cystatin C is combined with creatinine. Moreover, we found that the CKD-EPI equations that included cystatin C, when applied before donation, improved prediction of post-donation mGFR. The improvements were particularly pronounced in donors with high or low muscle mass, in whom pre-donation serum creatinine less strongly associated with pre- or post-donation mGFR. These results indicate an advantage for cystatin C (with or without creatinine) in potential living kidney donors, but the non-GFR determinants of cystatin C are less understood. Obesity, albuminuria, hypertension, diabetes, lower HDL cholesterol, higher triglycerides, higher C-reactive protein, higher uric acid and smoking have been described to influence cystatin C, but the pathways remain unclarified.[23–26] Hence, it is not clear when cystatin C-based eGFR should be interpreted with caution. It could be that these determinants are less influential for GFR estimates in healthy individuals than in disease populations, but further studies are needed to better understand this. Also, it has been shown that cystatin C is not totally independent of muscle mass[27,28], since muscle mass consists of nucleated cells and cystatin C is produced by all nucleated cells, but, variation in muscle mass has much less impact on cystatin C than on serum creatinine.

A new equation to predict post-donation mGFR

Living kidney donors compensate post-donation GFR up to 65-75% of the pre-donation value. Yet, predicting post-donation mGFR is not as simple as subtracting one third from the pre-donation GFR value, as demonstrated in **Chapter 4**.[29] We showed that some donors lose even 50% or more of their pre-donation eGFR, sometimes even when pre-donation eGFR is >100 mL/min/1.73m^2. Therefore, we developed an equation that predicts three months post-donation mGFR based on pre-donation age, sex and serum creatinine in **Chapter 4**. This equation performed better at predicting post-donation mGFR than subtracting 33% from pre-donation GFR, which was confirmed in internal and external validation cohorts. The equation could aid clinicians to identify which donors are at risk of low post-donation mGFR as well as to counsel the donor on the predicted post-donation GFR value. Unfortunately, we did not have sufficient cystatin C measurements available in the development, internal and external validation cohorts to investigate whether cystatin C could have improved our equation. The increase in predictive capacity (R^2) of the multivariable linear regression

model including age, sex, creatinine and cystatin C in **Chaper 3** suggests that improvement of the equation with cystatin C is likely. It is also important to mention that the equation was developed and validated in donors that were accepted for donation and actually donated, which may hamper applicability to potential donors with more extensive risk factors. Despite this limitation, we show that performance of the equation does not decline in subgroups with low pre- or post-donation mGFR, with a negative post-donation GFR slope or in a subgroup of older donors, but predictions should be interpreted with caution if a potential donor carries many risk factors.

Suggestion for clinical implementation

Based on the results of **Chapter 2, 3 and 4**, we would suggest to use the CKD-EPI 2021 equation that includes both creatinine and cystatin C to estimate kidney function routinely in all potential living kidney donors. Our equation from **Chapter 4** could be used as a supporting tool to predict post-donation mGFR, without the need to perform additional laboratory tests. When doubt exists whether pre-donation eGFR (CKD-EPI 2021 with creatinine and cystatin C) is acceptable, or whether predicted post-donation mGFR is acceptable, we suggest referral to a center that has mGFR available for confirmatory testing, as summarized in **Figure 1**. Future studies that include larger sample sizes should investigate whether our equation to predict post-donation mGFR from **Chapter 3** could be improved with addition of cystatin C.

Figure 1. Proposal for GFR assessment in potential living kidney donors based on this thesis.

The interpretation of hematuria in donor screening

Studies in **Chapter 2, 3 and 4** mainly focused on how to assess glomerular filtration rate as measure of kidney function, but in **Chapter 5** of this thesis, we studied microscopic hematuria as parameter of glomerular health.[30] Since erythrocytes in urine with no evidence for urological problems are likely indicative of a glomerular problem, international living kidney donor guidelines advise to perform a kidney biopsy in potential donors with microscopic hematuria without urological cause.[31,32] We know from experience that transplant centers rarely perform biopsies in potential donors with microscopic hematuria, as has been the

case in the UMCG. Our aim was to evaluate the consequences of refraining from kidney biopsy. We showed that donors with microscopic hematuria (≥3 red blood cells per µL or ≥1 red blood cell per high-power field) at donor screening do not display progressive worsening of eGFR, systolic blood pressure or proteinuria in the first five years after donation. It is important to emphasize that our results do not support acceptance of donors with microscopic hematuria without further assessment. Yet, these results could open the way for discussion about the role of kidney biopsies in potential donors with hematuria. Possibly, microscopic hematuria represents glomerular disease only in a small subgroup of otherwise healthy individuals. Future studies should explore alternative methods to identify these individuals, thereby preventing invasive kidney biopsies in those who are unlikely to have glomerular disease. Such tests may include analysis of red blood cell morphology. For example, Kido et al. distinguished between dysmorphic and normal red blood cells in donors with hematuria before donation and found that only hematuria with dysmorphic blood cells was associated with kidney function decline and proteinuria after donation.[33] Alternatively, genetic testing for Alport syndrome or thin basement membrane nephropathy may be considered in donors with first degree relatives with genetic kidney disease or kidney disease with unknown etiology, as proposed recently.[34,35]

Challenges in living kidney donor research

In **Part A** we studied methods to assess glomerular function and health in potential kidney donors and developed an equation to predict post-donation kidney function. Although the studies may improve pre- and post-donation kidney function assessment, they also share some limitations that bring forward challenges for future studies in the field. Since these challenges may apply to most studies in the field of living kidney donation, they are important to address. First, as a matter of course, we could only investigate post-donation risks in donors that were accepted for donation, causing potential selection bias. For example, in **Chapter 5**, we could not study post-donation outcomes in donors that were declined with microscopic hematuria. Similarly in **Chapter 3**, selection bias may make our equation less accurate in donors with many risk factors. Second, absence of a healthy control group with similar follow-up makes it difficult to determine to which extent the donation procedure contributed to certain outcomes (also referred to as the donation attributable risk).[36] Conducting a randomized controlled trial in which potential donors are assigned to a “treatment arm” (donation) and a “placebo arm” (no donation) is both unethical and unfeasible, but prospective cohort studies that also include a healthy control group are needed to better quantify the donation attributable risk. In order to get an idea on what

would have happened if declined donors had donated a kidney, it could be interesting to learn from less healthy populations that undergo unilateral nephrectomy outside the setting of living kidney donation, as we aimed to do in **Chapter 9**. These populations may carry more risk factors and could contribute to a better understanding of how risks factors like low kidney function, hypertension, diabetes or microscopic hematuria or other diseases impact post-nephrectomy outcomes. This especially interesting because selection criteria for living kidney donors tend to liberalize as a consequence of shortage of donor kidneys.[8]

Another limitation is the absence of follow-up beyond ten years post-donation. This is particularly important because CKD and kidney failure usually develop over years or even decades and manifest mostly later in life.[3] The few studies that report on >20 years of follow-up were mostly done in a cohort of ca. 4,000 living kidney donors from Minnesota.[37] In this cohort, 39 donors developed kidney failure at a mean of 27 years after donation, of which most of the known causes were hypertension and/or diabetes.[37] The rarity of outcomes as ESKD and mortality in living kidney donors, as shown in these studies, hampers research on kidney outcomes in the donor population. Alternatively, softer outcomes could be chosen, such as slopes of glomerular filtration rate (GFR), GFR below a certain threshold, development of albuminuria, hypertension and/or diabetes or cardiovascular disease. However, defining clinically relevant outcomes based on trends in kidney function and choosing appropriate statistical analysis methods can be challenging in observational studies.[38,39] This is particularly challenging in living kidney donors, since normal ranges of single-kidney GFR, and how they relate to ultra-long-term (decades) risk of kidney failure, are unknown.

PART B

Microstructural parameters as measure of kidney health

The glomerular filtration rate is usually not affected by minor kidney damage, because the remaining healthy nephrons are able to increase single-nephron GFR to compensate for nephron loss. We investigated whether microstructural parameters could serve as additional prognostic markers besides GFR to assess post-donation risks. It has been shown that living kidney donors with risk factors for CKD such as hypertension, overweight or a family history of kidney failure have higher glomerular volume in pre-implantation biopsies.[40] We confirmed these findings in **Chapter 7**, in which we found that glomerular volume was positively associated with body mass index, body surface area, waist-to-hip-ratio and blood pressure. Additionally, glomerular volume associated positively with

pre- and post-donation mGFR. In the context of hyperfiltration, we expected to find lower post-donation mGFR in donors with larger glomeruli, as was also the case in previous studies.[41,42] A possible explanation for this discrepancy could be that in **Chapter 7**, we used unindexed GFR in order to study actual filtration, whereas in other studies GFR was indexed for BSA. When we indexed mGFR for body surface area (data not shown in **Chapter 7**), the association between glomerular volume and pre- and post-donation mGFR disappeared, indicating that glomerular volume might be an effect mediator in the association between body surface area and mGFR. Another explanation for the discrepancies between our study and the Mayo Clinic study[43] could be differences in donor characteristics between the Mayo Clinic donor population and our population or the relatively short follow-up in our study. As highlighted in **Chapter 6**, abnormal findings in pre-implantation biopsies may provide information about subclinical kidney damage before donation and thereby identify which donors might benefit from more intensive follow-up. However, the aforementioned studies mostly focus on glomerular function and morphology as indicator of kidney health. Peritubular capillary rarefaction is a broadly recognized phenomenon in (more advanced) CKD, and therefore we studied the prognostic value of peritubular capillary healthy kidney donors for post-donation outcomes in **Chapter 7**.

In this chapter, we studied the association between glomerular volume and peritubular capillary density and whether peritubular capillary density correlated similarly with clinical characteristics and GFR as glomerular volume. The kidney is highly dependent on its vascularization for both its function as well as its metabolic demand, which is why it gets 20% of the cardiac output.[44] Kidney disease is characterized by peritubular capillary rarefaction, but also in age-related nephron loss, the number of peritubular capillaries is strongly correlated with glomerular and interstitial scarring.[44,45] It is unknown whether changes in glomerular morphology in healthy kidneys also correlate to PTC density. Especially because nowadays more donors with (well-controlled) hypertension are being accepted for donation, a disease in which capillary rarefaction can be both a cause as a consequence of the disease[45,46], living kidney donors might have varying PTC density. Moreover, increased glomerular volume accompanied with increased glomerular plasma flow as well as tubular hypertrophy and increased tubular metabolic demand might affect the peritubular capillaries resulting in either angiogenesis or capillary rarefaction. However, although we confirmed associations of glomerular volume with hypertension, body size and mGFR, we did not find a clear relation of either of these variables with peritubular capillary density. Even though this study had a relatively small sample size, the results suggest no role

for PTC density as marker for subclinical kidney damage in living kidney donors. While, unfortunately, this study did not reveal microstructural parameters that could be used in addition to GFR to assess kidney health, further studies are needed to better understand the subclinical alterations in kidney tissue that may indicate impaired kidney health before GFR decline ensues.

PART C

Hyperfiltration in remnant nephrons

The compensatory increase in GFR after unilateral (donor) nephrectomy commences directly after unilateral donor nephrectomy and continues up to five years.[10] The rise in GFR is achieved by increasing single-nephron GFR and as a result, glomeruli enlarge jointly with total cortical volume.[47,48] The exact mechanisms behind the compensatory GFR increase after living kidney donation are not fully understood. In 1981, Hostetter *et al.* found that the increase in single-nephron GFR after 5/6 nephrectomy in rats was most likely the result of increased kidney plasma flow and hydraulic pressure gradient.[49] Structural alterations in the glomeruli observed in the nephrectomized rats could largely be prevented by low-protein diet, supporting the role of increased kidney plasma flow and intraglomerular pressure in damaging the glomerulus.[49,50] These studies formed the basis for the "Brenner hypothesis": that substantial loss of nephrons, independent of the cause, leads to a final common pathway of glomerular hyperfiltration in remaining nephrons due to increased glomerular plasma flow and intraglomerular pressure, resulting in glomerular damage, increased glomerular permeability and subsequent proteinuria.[49,51,52] This hypothesis has raised concerns for kidney donors as removal of 50% of nephrons may put donors at higher risk of glomerular hyperfiltration with subsequent glomerular damage and loss of kidney function. Yet, increased glomerular pressure does not seem to be the sole mechanism by which glomeruli enlarge, as demonstrated by the inhibition of compensatory kidney growth after administering a growth hormone receptor antagonist.[53] Also, in the 5/6-nephrectomy rat models 90% of the functioning nephrons were removed, thereby practically inducing kidney failure.[49] The question is whether these results are applicable to settings in which a smaller proportion of functioning nephrons is removed, especially since the extent of glomerular hypertrophy and hyperfiltration strongly correlates with the amount of kidney mass removed.[54,55] Additionally, Chamberlain *et al.* demonstrated that removal of 50% of functioning nephrons resulted in a quick and strong short-term GFR increase, whereas removal of 90% resulted in a slower and less strong GFR increase.[56] Also, 90% removal resulted in a post-nephrectomy fractional

water-, sodium- and potassium excretion course that strongly deviated from the fractional excretions in sham-operated rats.[56] When only 50% of kidney mass was removed, the post-nephrectomy courses of fractional water-, sodium- and potassium excretion were more comparable to sham-operated rats.[56] These results suggest that compensatory mechanisms may differ according to the proportion of functioning nephrons that are removed.

Post-donation hyperfiltration and long-term outcomes

It is currently unclear whether hyperfiltration in the remnant nephrons is responsible for the small but increased risk of living kidney donors to develop kidney failure. Some studies report an increased risk of elevated proteinuria and/or albuminuria after living kidney donation[57,58], but others report no differences between donors and healthy controls[59], and none of them report accelerated GFR decline in living kidney donors.[57–59] Also, the studies that report long-term kidney failure risk describe diabetes[60], hypertension[60,61], glomerulonephritis[62], or primary kidney disease[63] as most common reasons for kidney failure, pointing away from nephrectomy-induced glomerular hyperfiltration as primary etiology. Kasiske *et al.* found stable courses of kidney function, no increased urinary protein or albumin excretion compared to contemporary healthy controls, but they found some vascular and metabolic differences to the detriment of kidney donors that could lead to future adverse outcomes.[59] Altogether, there is no evidence in currently existing literature that hyperfiltration itself in the remnant nephrons of kidney donors is responsible for adverse kidney function outcomes. For this reason, in **Chapter 8**, we studied whether short-term post-donation hyperfiltration in the remaining kidney predicted long-term kidney function or proteinuria.[64] We defined short-term hyperfiltration as the difference between the three months post-donation single-kidney GFR and the pre-donation single-kidney GFR, which was calculated as 50% of total pre-donation GFR (**Figure 2**). We found that a stronger increase at three months, indicating more hyperfiltration in the remaining nephrons, predicted better five and ten years GFR, independent of pre-donation GFR and age. Replication of our analyses in an independent cohort yielded similar results and results were also consistent for eGFR and mGFR. Additionally, in order to account for potential differences in left and right kidney volume, we adjusted our calculation of the increase in single-kidney GFR for the cortical volume of the removed kidney (assessed by CT scan), which did not affect our results. Moreover, the increase in single-kidney GFR was not associated with the protein/creatinine ratio at five or ten years after donation. These results suggest no adverse effects of post-nephrectomy hyperfiltration in the remaining nephrons after unilateral donor nephrectomy. The

conclusions of Lenihan *et al.* that compensatory hyperfiltration remains stable for six to eight years after donation and is achieved by a rise in plasma flow and ultrafiltration coefficient rather than glomerular hypertension support our findings.[48] Even though we had no follow-up beyond ten years, we found not even a small indication of adverse outcomes in donors with stronger hyperfiltration. Taking in mind that the donors had already been exposed to this hyperfiltration for 10 years, we do not expect that progressive kidney function decline due to hyperfiltration starts after ten years, but this needs to be confirmed in studies with longer follow-up. Also in future studies, associations with other outcomes such as new-onset hypertension, cardiovascular disease, alterations in bone- and mineral metabolism and hyperuricemia and gout should be explored.[65]

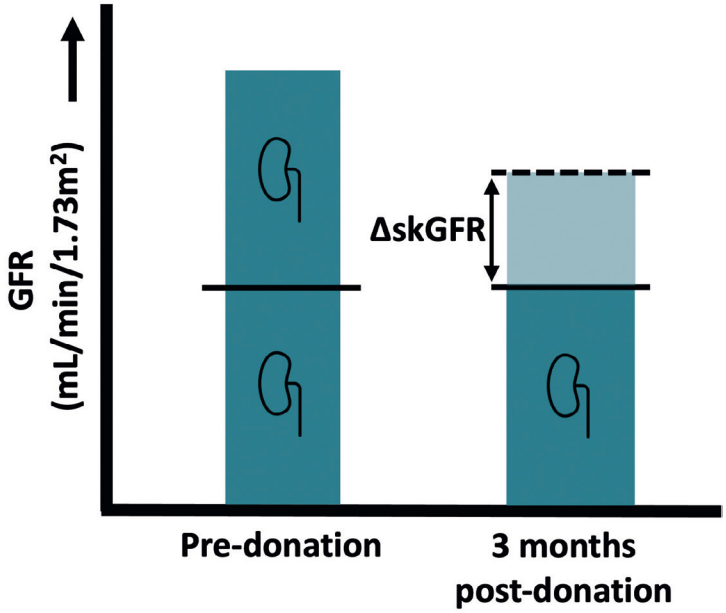

Figure 2. Schematic representation of kidney function in the pre- (two kidneys) and post-donation (one kidney) setting.

The light blue part represents the compensatory increase in GFR by the remaining kidney (delta single kidney-GFR (Δsk-GFR)).

The reserve capacity

Based on the results of **Chapter 8**, we hypothesized that that a larger increase in single-kidney GFR after unilateral nephrectomy might be an expression of greater resilience rather than an indication of malignant hyperfiltration. This

post-donation compensatory response might be driven by the reserve capacity in the kidney, which might explain why the extent of compensation varies between individuals. Possibly, donors with less compensatory increase in single-kidney GFR were subject to nephron loss prior to donation for which the reserve capacity was used in the remaining nephrons to maintain GFR, resulting in less compensation post-donation. This is supported by our findings in **Chapter 8** that both age and body surface area were inversely associated with the post-nephrectomy increase in single-kidney GFR.[64] Additionally, pre-donation eGFR and cortical kidney volume were positively associated with the post-nephrectomy increase in single-kidney GFR. Even though we identified several independent determinants that were consistent in the replication cohort, the total variance in short-term increase in single-kidney GFR explained by our model did not exceed 20%, indicating that the majority of factors driving post-donation compensation remain unknown.

Hyperfiltration in other populations

In the past decades, living kidney donor characteristics have changed.[8,66] Donors nowadays are older, more overweight and more frequently accepted for donation with (well-controlled) hypertension. These changes potentially impact the reserve capacity of the kidney, resulting in either 1) less compensatory hyperfiltration or 2) hyperfiltration to a similar extent but accompanied by increased intraglomerular pressure leading to adverse outcomes according to the Brenner hypothesis discussed above.[52] In **Chapter 9** of this thesis, we studied the prognostic value of short-term post-nephrectomy hyperfiltration for long-term kidney function in a less healthy population that underwent nephrectomy outside the setting of donation, mainly for kidney cancer. Interestingly, the short-term post-nephrectomy increase in GFR followed the same distribution as in the living kidney donor population and, of note, a stronger short-term increase in single-kidney GFR was independently associated with a lower risk of 30% decline in eGFR upon follow-up. Again, we found no indication that stronger hyperfiltration after unilateral nephrectomy results in progressive kidney function decline. The patients in **Chapter 9** were clearly less healthy than the donors in **Chapter 8**, illustrated by the number of events and number of deaths during follow-up accounting for one third of the total population. This in comparison to **Chapter 8**, where none of the donors experienced a 30% decline in eGFR and the number of deaths was minor and never kidney disease related. Whether hyperfiltration in remnant nephrons played a role in the patients with events in **Chapter 9** remains unanswered, but we showed that the magnitude of hyperfiltration is not an indicator of worse kidney function outcomes. Future studies should in-

vestigate differences between potential benign and pathological mechanisms of post-nephrectomy hyperfiltration and how they could be distinguished. The positive association between cortical kidney volume and post-donation adaptation of GFR that we found in **Chapter 8** suggests a potential explanatory part for nephron number. Low nephron number at birth is associated with development of CKD and kidney failure later in life, and possibly also a reduced reserve capacity.[67] However, studying the role of nephron number warrants invention of easy and non-invasive methods to accurately estimate nephron number that currently do not (yet) exist. This would also be relevant for other settings in which the reserve capacity might play a role, for example in recovery after acute kidney injury (AKI). It is well-known that stronger recovery of GFR after AKI is associated with better long-term outcomes[68–71], but in absence of knowledge on how many nephrons were lost it remains unknown who recovers at the expense of the reserve capacity.

Conclusions and future directions

The aims of this thesis were to explore new and existing methods to assess pre-donation glomerular health in potential living kidney donors (**Part A**), to study microstructural variations in healthy donor kidneys and their relation with pre- and post-donation kidney function (**Part B**) and to study the prognostic value of post-donation compensatory hyperfiltration (**Part C**). Based on our studies in **Part A**, we propose a strategy for kidney function assessment in potential living kidney donors. We showed that benefits of routine use of mGFR are likely minimal and found that the CKD-EPI 2021 equation based on both creatinine and cystatin C calculated before donation was most accurate to predict pre- and post-donation mGFR. Therefore, we suggest to use this equation in routine donor evaluation. Additionally, we developed an equation based on pre-donation serum creatinine, age and sex that predicts three months post-donation mGFR, which could be used as a supporting tool in donor evaluation and counseling. Future studies should investigate whether this equation could improve with addition of cystatin C. Next, we investigated whether subclinical alterations in kidney tissue on microstructural level could be prognostic for post-donation kidney function, in addition to pre-donation GFR in **Part B**. Although we could confirm associations of glomerular volume with GFR, hypertension and body size in line with prior studies, we found no associations of peritubular capillary density with glomerular volume, nor with kidney function or donor characteristics. For now, the prognostic value of parameters obtained at biopsy seems limited for living kidney donors. Further studies are needed to better understand microstructural alterations in relation to kidney health and the potential prognostic role in living

kidney donors. Lastly, in **Part C**, we found that more short-term post-nephrectomy hyperfiltration, defined as larger compensatory GFR increase above 50% of the pre-donation value is an independent marker of better long-term kidney function. We hypothesized that a larger compensatory GFR increase may be an expression of more reserve capacity in the remaining kidney. In future studies, differences between potential benign and malignant nephron hyperfiltration should be investigated. Also, driving mechanisms of the reserve capacity, such as nephron number at birth, should be studied. Finally, establishing non-invasive methods to accurately estimate nephron number and renal reserve without requiring a nephrectomy would be essential, as these may have additional clinical value when combined with kidney function itself. Together, these future developments will pave the way for further improvement of kidney health assessment in living kidney donors and beyond.

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DUTCH SUMMARY

Nederlandse samenvatting

Levende nierdonoren worden uitgebreid geëvalueerd op gezondheid voordat zij een nier mogen afstaan. Één van de belangrijkste onderdelen van deze evaluatie betreft het inschatten of de nieren van de donor voldoende functioneren om in goede gezondheid met één nier verder te leven. De best beschikbare maat voor nierfunctie is de glomerulaire filtratie snelheid ("glomerular filtration rate", GFR): een maat die uitdrukt hoeveel plasma door de glomeruli gefiltreerd wordt per tijdseenheid, meestal gecorrigeerd voor lichaamsoppervlakte (mL/min/1.73m^2). De GFR kan op verschillende manieren bepaald worden. De gemeten GFR ("measured GFR", mGFR) wordt gezien als de gouden standaard, en wordt bepaald door een radioactief gelabelde marker intraveneus toe te dienen, waarna over een bepaalde tijdsperiode plasma en urine concentraties van de marker worden gemeten. Met deze concentraties kan berekend worden hoeveel de nieren per tijdseenheid uitscheiden in de urine. Een nadeel van deze methode is dat het veel tijd kost en duur is en daarom niet geschikt om op grote schaal in de kliniek toe te passen. De GFR kan ook geschat worden ("estimated GFR", eGFR) op basis van plasmaconcentraties van lichaamseigen markers, zoals plasma creatinine of cystatine C. Dit zijn beide markers die op een constante snelheid door het lichaam worden geproduceerd en in principe alleen door de nieren worden uitgescheiden. Omdat ze op constante snelheid worden geproduceerd worden de plasmaconcentraties voornamelijk beïnvloed door veranderingen in de nierfunctie. Er zijn verschillende vergelijkingen beschikbaar om de GFR schatten op basis van plasma creatinine en/of cystatine C, waarin ook gecorrigeerd wordt voor non-GFR determinanten van deze markers, zoals geslacht en leeftijd. De CKD-EPI vergelijking is de meest gebruikte variant en kent vijf versies gebaseerd op creatinine en/of cystatine C die over de jaren heen ontwikkeld zijn. Het probleem van deze vergelijkingen is echter dat ze ontwikkeld zijn in andere populaties (met een lagere GFR) en daardoor de nierfunctie in nierdonoren vaak onderschatten.

In **Deel A** van dit proefschrift hebben wij nieuwe en bestaande methoden om nierfunctie in potentiële levende nierdonoren te bepalen onderzocht. Op basis hiervan hebben wij een voorstel gedaan voor een strategie om nierfunctie te bepalen tijdens de evaluatie van potentiële nierdonoren. Als eerste hebben wij in **Hoofdstuk 2** gekeken of een mGFR meting doen in iedere potentiële donor kandidaat zou kunnen leiden tot het accepteren van meer donoren. De hypothese hierachter was dat door de systematische onderschatting van de nierfunctie door eGFR, donoren mogelijk eerder worden afgewezen op basis van een

lage GFR als de eGFR wordt gebruikt dan wanneer de mGFR wordt gebruikt. Wij zagen echter dat de gemiddelde eGFR voor donatie niet lager was in een centrum waar de beslissing om donoren te accepteren werd gebaseerd op de mGFR dan in centra waar die beslissing werd gebaseerd op de eGFR. Daarom hebben wij geconcludeerd dat het routinematig toepassen van de mGFR in potentiële donoren waarschijnlijk geen voordelen oplevert, zeker niet in relatie tot de financiële en praktische nadelen van deze methode. Vervolgens hebben wij in **Hoofdstuk 3** gekeken welke van de CKD-EPI vergelijkingen het meest nauwkeurig de GFR schat in potentiële donoren. Hierbij hebben wij ook gekeken naar de voorspellende waarde van de pre-donatie CKD-EPI-gebaseerde eGFR voor post-donatie mGFR. Hoewel de creatinine-gebaseerde CKD-EPI op dit moment het meest gebruikt wordt voor donor evaluatie, werd in **Hoofdstuk 3** duidelijk dat de CKD-EPI vergelijkingen die zowel op creatinine als cystatine C zijn gebaseerd het meest nauwkeurig zijn voor het schatten van de pre-donatie GFR. Dit gold ook voor het voorspellen van de post-donatie mGFR. De verbetering door het combineren van beide markers werd met name duidelijk in een subgroep donoren het een hoge of lage spiermassa (bepaald door de 24-uurs creatinine uitscheiding). In deze subgroep deed de creatinine-gebaseerde CKD-EPI vergelijking het aanzienlijk slechter, waarschijnlijk omdat plasma creatinine concentraties (een afbraakproduct van spiermetabolisme) zeer sterk beïnvloed worden door spiermassa.

In **Hoofdstuk 4** hebben wij onderzocht of het voordelig zou kunnen zijn om een specifieke donor vergelijking te ontwikkelen voor het schatten van de nierfunctie. Omdat het grootste doel van de nierfunctiebepaling voor donatie is om in te schatten of de donor voldoende nierfunctie overhoudt na de donatie, hebben wij een formule ontwikkeld die op basis van pre-donatie plasma creatinine, leeftijd en geslacht de post-donatie GFR voorspelt. Deze formule zou als ondersteunend middel gebruikt kunnen worden tijdens de evaluatie van levende donoren. De arts heeft op deze manier meer informatie over de voorspelde restnierfunctie, en de donor kan beter geïnformeerd worden over de voorspelde nierfunctie na donatie. Op basis van **Hoofdstuk 2, 3** en **4** zouden wij dan ook adviseren om de nierfunctie in potentiële donoren te schatten op basis van de CKD-EPI vergelijking die zowel creatinine als cystatine C bevat. Wegens ethische bezwaren tegen de CKD-EPI 2012 vergelijking, die zowel creatinine als cystatine C bevat maar ook een coëfficiënt voor ras heeft, heeft het gebruik van de CKD-EPI 2021 vergelijking gebaseerd op beide markers de voorkeur. Daarnaast kan voor het voorspellen van de post-donatie GFR onze formule uit **Hoofdstuk 4** gebruikt worden. Wanneer twijfel bestaat over of de pre-donatie of voorspelde post-do-

natie GFR voldoende is, adviseren wij om te verwijzen naar een centrum waar de mGFR methode beschikbaar is.

Naast het bepalen van de GFR zijn er meer manieren om een indruk te krijgen van de gezondheid en/of het functioneren van de nier. Dit wordt onder andere gedaan door de urine te screenen op aanwezigheid van rode bloedcellen (hematurie). Omdat rode bloedcellen in de urine die niet door een urologisch probleem verklaard kunnen worden waarschijnlijk uit de glomerulus afkomstig zijn, adviseren de internationale donor richtlijnen om een biopt te doen in zulke gevallen. Microscopische hematurie komt in 8-20% van de normale bevolking voor en buiten de donatie setting zou er bij asymptomatische microscopische hematurie normaal gesproken geen biopt gedaan worden, omdat de prognose gunstig is. Dit in combinatie met het feit dat een biopt een invasieve procedure is die niet risicovrij is zorgt ervoor dat deze biopten zelden tot nooit worden gedaan tijdens donorkeuringen in transplantatiecentra, ondanks de aanbeveling in de richtlijnen om dit wel te doen. In **Hoofdstuk 5** hadden wij als doel om de consequenties van het niet doen van deze biopten in donoren met microscopische hematurie te evalueren. Hierbij zagen wij dat donoren met microscopische hematurie voor donatie geen slechter beloop van nierfunctie na donatie hebben dan donoren zonder hematurie. Ook hadden zij geen hogere bloeddruk en ontwikkelden ze niet sneller proteïnurie. Ondanks dat deze resultaten geen reden zijn om vanaf nu altijd donoren met hematurie te accepteren zonder biopt, denken wij wel dat dit hoofdstuk een reden geeft om de rol van biopten in donorkeuringen te hereva-lueren. Mogelijk is er alleen in een subgroep van donoren met microscopische hematurie sprake van een glomerulair probleem. Wij denken dat het belangrijk is om opzoek te gaan naar methoden die deze mensen zouden kunnen identificeren, zoals rode bloedcel morfologie of genetisch testen op Alport syndroom of dunne membraan ziekte. Op deze manier zou de groep waarbij een biopt geïndiceerd is kleiner gemaakt kunnen worden.

Het is bekend dat de nier beschikt over compensatiemechanismen waardoor in het geval van schade of verlies van nefronen, de filtratiesnelheid in de resterende nefronen omhooggaat, wat ervoor zorgt dat de totale GFR constant blijft. Hierdoor is de GFR niet altijd een perfecte reflectie van het aantal functionerende nefronen in de nier, en blijft subtiele schade vaak onopgemerkt. In **Deel B** van dit proefschrift hebben wij daarom onderzocht of het analyseren van microstructurele parameters in biopten van levende nierdonoren aanvullende prognostische informatie zou kunnen geven voor post-donatie uitkomsten. Uit eerdere studies, zoals ook besproken in **Hoofdstuk 6**, blijkt dat glomerulaire hypertrofie geas-

socieerd is met risicofactoren voor chronische nierziekte (zoals hypertensie en overgewicht) als ook met een lagere korte- en lange termijn nierfunctie na donatie. In **Hoofdstuk 7** konden wij de associaties tussen glomerulaire hypertrofie en hypertensie en overgewicht bevestigen, maar vonden wij juist een positieve correlatie met post-donatie nierfunctie. In **Hoofdstuk 7** hebben wij, naast glomerulaire hypertrofie, ook gekeken naar de densiteit van peritubulaire capillairen als mogelijke maat voor subtiele nierschade. Het is bekend dat verlies van peritubulaire capillairen een belangrijke rol speelt in de progressie van chronische nierziekten. Zo is de peritubulaire capillaire densiteit een voorspellende factor voor nierfunctie in niertransplantatiepatiënten, maar of de peritubulaire capillaire densiteit ook gerelateerd is aan nierfunctie in gezonde nieren is niet bekend. Ondanks dat we de associatie tussen glomerulaire hypertrofie met risicofactoren voor nierziekten uit eerdere studies konden bevestigen, vonden wij geen relatie tussen peritubulaire capillaire densiteit en klinische karakteristieken of nierfunctie. Daarom hebben wij geconcludeerd dat deze marker voor nu waarschijnlijk geen additionele prognostische waarde lijkt te hebben in nierdonoren, maar om dit beter te begrijpen zijn meer studies in grotere groepen nodig.

De capaciteit van de nier om te compenseren voor het verlies van nefronen komt ook na de donatie tot uiting. Ondanks dat 50% van de niermassa wordt verwijderd (één nier), houdt een donor ongeveer 65-75% van de nierfunctie over na donatie. Omdat er geen nieuwe nefronen gevormd kunnen worden in de nier, gebeurt dit door het toenemen van de glomerulaire filtratiesnelheid in de resterende nefronen. Uit experimentele studies in ratten is gebleken dat het verwijderen van 5/6 deel van de niermassa ervoor zorgt dat de resterende nefronen dusdanig gaan hyperfiltreren dat er schade aan de glomeruli ontstaat, leidend tot proteïnurie en een verdere verslechtering van de nierfunctie. Door deze studies was het niet altijd duidelijk of de compensatoire toename in nierfunctie na donatie een goed teken was of dat het zorgde voor hyperfiltratie die op de lange termijn tot meer schade zou kunnen leiden. Echter, de goede uitkomsten in levende donoren doen vermoeden dat de rol van schadelijke hyperfiltratie na donatie mogelijk beperkt is. Dit hebben wij onderzocht in **Deel C** van dit proefschrift. In **Hoofdstuk 8** hebben we gevonden dat meer compensatoire toename in GFR na donatie (gedefinieerd als de toename van GFR boven 50% van de pre-donatie GFR) een onafhankelijke voorspeller is voor een betere GFR vijf en tien jaar na donatie. Ook vonden wij geen associatie tussen de compensatoire toename in GFR en het ontstaan van proteïnurie. Onze hypothese is daarom dat een sterkere toename in GFR na donatie mogelijk een uiting is van veerkrachtigheid, mogelijk gedreven door de reservecapaciteit van de nier. Omdat nierdonoren een zeer

gezonde populatie zijn waarbij het risico op slechte uitkomsten laag is, wilden wij dit ook onderzoeken in een minder gezonde populatie. Daarom hebben wij voor **Hoofdstuk 9** samengewerkt met het Karolinska Instituut in Stockholm. Het Karolinska Instituut beschikt over een database (Serum CREAtinine Measurement (SCREAM) project) die alle labwaarden, diagnosecodes en voorgeschreven medicatie bevat van mensen in de regio van Stockholm. Uit deze database hebben wij patiënten geselecteerd die een nefrectomie hebben ondergaan voor een andere reden dan nierdonatie (voornamelijk vanwege nierkanker). Deze mensen hebben meer risicofactoren voor nierschade (bijvoorbeeld diabetes, hypertensie of gebruik van nefrotoxische medicatie), waardoor hun reservecapaciteit mogelijk verminderd is. Ook in deze populatie hebben wij gevonden dat meer compensatoire toename in GFR op de korte termijn na de nefrectomie een onafhankelijke voorspeller was voor een beter beloop van GFR op de lange termijn. Uit de studies in **Hoofdstuk 8** en **9** hebben wij daarom geconcludeerd dat de mate van compensatoire toename in GFR na nefrectomie geen indicatie is voor slechtere uitkomsten op de lange termijn. Mogelijk komt dit doordat de nier beschikt over een reservecapaciteit die de nier in staat stelt te compenseren voor verlies van nefronen, zonder schade toe te brengen aan de resterende nefronen. In de toekomst is het belangrijk om te ontdekken hoe de reservecapaciteit gemeten kan worden, zodat donoren die risico lopen op weinig compensatie na donatie geïdentificeerd kunnen worden.

Concluderend is in dit proefschrift het bepalen van de gezondheid van de nier in levende nierdonoren onderzocht in die tijdsperken rondom levende nierdonatie: voor de donatie (**Deel A**), tijdens de transplantatie (**Deel B**) en na de donatie (**Deel C**). Er is een voorstel gedaan voor een strategie om de nierfunctie voor donatie te bepalen in **Deel A**. De in **Deel B** onderzochte peritubulaire capillaire densiteit lijkt voor nu geen additionele prognostische waarde te hebben voor levende donoren. Tot slot hebben wij op basis van de resultaten in **Deel C** een hypothese gegenereerd over dat post-donatie compensatie van GFR mogelijk gedreven wordt door de reservecapaciteit van de nier. Hierbij hebben wij suggesties gedaan voor toekomstig onderzoek dat zich moet richten op het meten van de reservecapaciteit. Niet alleen voor het selecteren van nierdonoren zal het nuttig zijn om de reservecapaciteit van de nieren te kunnen bepalen, maar ook in chronische- en acute nierziekte zou het relevant kunnen zijn om te weten hoeveel reservecapaciteit een nier heeft of heeft ingeleverd.

LIST OF PUBLICATIONS

Microscopic Hematuria at Kidney Donor Screening and Post-Donation Kidney Outcomes.
van der Weijden J, van Londen M, Pol RA, Sanders JF, Navis G, Nolte IM, de Borst MH, Berger SP.
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Impact of measured versus estimated glomerular filtration rate-based screening on living kidney donor characteristics: A study of multiple cohorts.
van der Weijden J, van Londen M, Roodnat JI, Kho ML, van de Wetering J, Kloke H, Dooper IMM, Bakker SJL, Navis G, Nolte IM, De Borst MH, Berger SP.
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Prediction of measured GFR after living kidney donation from pre-donation parameters.
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CURRICULUM VITAE

Jessica van der Weijden werd op 10 augustus 1995 geboren in Amsterdam. Op zesjarige leeftijd verhuisde zij naar Heemstede waar zij opgroeide en in 2013 haar vwo-diploma behaalde aan het Atheneum College Hageveld. Via de decentrale selectie werd zij datzelfde jaar aangenomen voor de studie geneeskunde aan de Rijksuniversiteit Groningen. In 2016 behaalde zij haar bachelor diploma en tijdens de wachttijd voor de master Geneeskunde vertrok zij samen met een (studie) vriendin voor drie maanden naar Kenia. Hier heeft zij onder begeleiding van professor Khama Rogo stagegelopen in het Sagam Community Hospital, gelegen in de buurt van Kisumu. Toen zij terugkwam in Nederland startte zij in februari 2017 met haar wetenschappelijke stage onder begeleiding van dr. M. van Londen, prof. M.H. De Borst en prof. G.J. Navis. Tijdens deze stage schreef zij haar masterscriptie over de voorspellende waarde van pre-donatie eGFR voor post-donatie mGFR in levende nierdonoren. Na deze stage startte zij met haar coschappen in het Martini Ziekenhuis in Groningen en later in het Isala Ziekenhuis in Zwolle. Tijdens haar coschappen werd zij aangenomen voor het MD/PhD-traject, waarin zij onder begeleiding van prof. S.P. Berger, prof. M.H. de Borst, dr. M. van Londen en dr. I.M. Nolte aan dit proefschrift werkte. Het traject werd met een half jaar verlengd om een onderzoeksproject te doen bij de afdeling Medische Epidemiologie en Biostatistiek aan het Karolinska Instituut in Stockholm, onder begeleiding van prof. J.J. Carrero. Na het afronden van haar promotietraject in maart 2023 is zij in juni 2023 gestart met haar semi-artsstage bij de Maag-, Darm- en Leverziekten in het Flevoziekenhuis en bij de Anesthesiologie in het Amsterdam Universitair Medisch Centrum, waarna zij zal afstuderen in december 2023.

DANKWOORD

Dit proefschrift is uiteraard niet het werk van mij alleen. Zonder de samenwerking met mijn promotieteam en collega's en de support van familie en vrienden zou dit werk niet tot stand zijn gekomen. Een aantal mensen zou ik daarvoor in het bijzonder willen bedanken.

Als eerste wil ik mijn dankbaarheid uitspreken dat ik heb mogen samenwerken met een geweldig team van begeleiding: **Stefan**, **Martin**, **Marco** en **Ilja**. Wat heb ik veel van jullie kunnen leren en wat ben ik dankbaar dat jullie er al die jaren voor mij zijn geweest. Jullie professionaliteit en support heeft ervoor gezorgd dat ik me nooit zorgen hoefde te maken over dat het niet goed zou komen.

Stefan, jouw passie voor je vak en de medische wetenschap is inspirerend. Van jou heb ik geleerd om altijd dicht bij de data te blijven, ook als ze in strijd zijn met de huidige klinische praktijk of richtlijnen of als er niet uitkwam wat er werd verwacht. Dat het juist leuk en belangrijk is om dit soort data te delen, en dat het vaak tot interessante discussies en nieuwe inzichten leidt. Bedankt dat je altijd de tijd nam om hierover met mij te filosoferen, maar bovenal bedankt dat je altijd vertrouwen in me hebt gehad en dit naar me uitsprak als ik dat niet had.

Martin, het zal wennen zijn om niet iedere woensdagochtend om 9:00 je kantoor (of Teams) meer binnen te stappen. Ik heb enorm genoten en veel geleerd van onze discussies over de reservecapaciteit van de nier en voel me vereerd dat ik een klein onderdeel mocht uitmaken van hoe jij dit concept de afgelopen jaren hebt uitgewerkt. Ik bewonder hoe je met jouw schrijfkunsten, oog voor detail en volhardendheid manuscripten altijd tot een beter geheel kunt maken. Daarnaast wil ik je bedanken voor je altijd aanwezige support (voor mijn eeuwige buitenland plannen, maar ook als ik door mijn eigen fout een subsidieaanvraag een dag te laat indiende).

Marco, ik denk dat we wel kunnen stellen dat zonder jou, dit proefschrift er helemaal niet geweest zou zijn. Onze samenwerking gaat terug naar 2015 en ik kan het niet vaak genoeg zeggen: ik gun iedereen een begeleider zoals jij. Jouw vertrouwen in mij was vanaf het begin aanwezig en heeft ervoor gezorgd dat ik in 2018 durfde te solliciteren voor het MD/PhD-traject. Ik ben je daar eeuwig dankbaar voor. Je maakte al-tijd tijd vrij om dingen te bespreken of uit te leggen – ook als het voor de tiende keer was (ode aan je tekeningen in paint!) – en je checkte regelmatig hoe het ging. Bij jou staan de wensen en ambities van je

studenten centraal en je bent een meester in enthousiasmeren. Jouw support, enthousiasme, intelligentie en creativiteit zijn echt uniek, en ik weet zeker dat deze eigenschappen je ver gaan brengen. Ik ben je in ieder geval enorm dankbaar voor de afgelopen jaren en hoop dat we onze samenwerking in de toekomst voort kunnen zetten.

Ilja, bedankt dat ik altijd bij je terecht kon met mijn vragen over statistiek. Met name de analyses uit hoofdstuk vijf had ik niet kunnen uitvoeren zonder jouw begeleiding en uitleg. Bedankt dat je je bij ons team wilde aansluiten om te waarborgen dat de statistiek juist uitgevoerd werd.

It had been my wish since the beginning of my MD/PhD training to spend some research time abroad. After many plans, Martin brought me in contact with Professor **Juan-Jesus Carrero**, who welcomed me at the department of Medical Epidemiology and Biostatistics (MEB), Karolinska Institutet Stockholm. Dear **JJ**, it has been a huge honor to have had the chance to work with you, **Faizan** and **Edouard** (mostly from Boston) on this project. Besides the fact that I learned a lot from you, I also had a great time with you and the research group. **Stefania**, **Alessandro**, **Ailema**, **Anne-Laure**, **Yuanhang** and **Faizan**, thank you all for the good times, laughs during lunch, as well as the fun dinners and going out for drinks. Last but not least: **Linn**, thank you for making me feel so welcome at MEB. I really hope to meet all of you again someday and I promise I won't make you cheese sandwiches ;).

Speaking of going abroad: I would like to express gratitude to Professor **Valerie Luyckx** for teaching a week-long scientific writing course and spending time together with all the students at Villa Camozzi. I feel lucky to have been able to participate in this course to improve my writing skills.

Stephan, **Gerjan** en **Robert**, ook al waren jullie niet officieel mijn begeleiders, kon ik altijd bij jullie terecht voor overleg of advies. Bedankt voor jullie waardevolle bijdrage aan de hoofdstukken van dit proefschrift en **Gerjan**, bedankt voor je hulp bij het oefenen voor mijn MD/PhD sollicitatie.

Dan zou ik ook nog graag mijn dank willen uitspreken naar mijn collega's van de nefro en chirurgie. Bedankt dat ik altijd bij jullie terecht kon met vragen, maar ook bedankt voor de leuke tijd, zowel in het UMCG als daarbuiten op congressen, promoties, vrijdagmiddagborrels, sinterklaasavonden, festivals en weekendjes weg. Een paar mensen wil ik graag in het bijzonder noemen. **Antonio**

en **Daan**, bedankt voor jullie hulp en ondersteuning bij het uittrekken en analyseren van TransplantLines data. **Lisa**, als enige mede donoren-PhD'er voelde jij altijd als mijn teamgenoot, bedankt voor de fijne samenwerking. Dames van de nierfunctiekamer: **Roelie**, **Dirkina** en **Marian**, bedankt voor jullie hulp bij de TransplantLines visites in de nierfunctiekamer. **Ineke**, bedankt voor je hulp bij het verzamelen van de data voor het hematurie stuk. **Evelien**, **Wiesje**, **Tamar** en **Annick**, bedankt voor de gezelligheid tijdens lunches in het UMCG, maar vooral voor de geweldige tijd die we hebben doorgebracht op congressen in Kopenhagen, Milaan en Buenos Aires.

Bedankt aan al mijn lieve vrienden en familie voor de support tijdens het schrijven van dit proefschrift. Bedankt dat ik weekend na weekend bij jullie in Amsterdam mocht logeren tijdens de 4 jaar dat ik langer in Groningen bleef dan jullie. Lieve **Ies** en **Wieb**, ik kan me geen fijnere plek bedenken om je proefschrift af te ronden dan waar ik nu met jullie woon. Bedankt dat jullie mij met open armen ontvingen. Natuurlijk ook bedankt aan mijn lieve huisgenoten **Bente**, **Wiesje**, **Ineke** en **Isabel**, dankzij jullie bleef Groningen óók als thuis voelen. Lieve **pap**, **mam**, **Bas** en **Anne**, uiteraard ook bedankt voor jullie liefde en support. Zonder jullie zou ik nooit staan waar ik nu sta. **Mam**, in het bijzonder bedankt voor je support in de aanloop naar dit traject, dankzij jou worden alle beren op de weg altijd weer klein.

Lieve **Lars**, allereerst natuurlijk bedankt voor het design van de buitenkant van dit proefschrift, ik ben er super blij mee. Bedankt voor je oneindige support en liefde, ik ben blij dat jij in mijn leven bent en dat ik dit met jou kan delen.

Tot slot bedankt aan mijn paranimfen, **Venla** en **Stefanie**, dat jullie naast mij willen staan op deze dag. **Ven**, we hebben het hele MD/PhD-traject van begin tot eind samen meegemaakt. Naast onderzoek hebben we een geweldige tijd gehad, samen met **Pritt** en **Rox**, toen we samenwoonden in ons huis (De Cave) in Zwolle, ondanks de bizarre tijd met alle lockdowns. Het was een feest om met je te wonen en ik ben dankbaar dat dankzij ons MD/PhD-traject deze vriendschap is ontstaan. **Stef**, ik was samen met jou op reis toen ik besloot om onderzoek te gaan doen. We hebben de hele bachelor samen doorlopen: samen in de collegebanken, hetzelfde UB-ritme, Wok To Go voor ieder tentamen en samen naar de kroeg als het er weer op zat. Onze vriendschap is me heel dierbaar en ik ben blij dat je naast me wil staan op deze dag!