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ORIGINAL ARTICLE

Impact of errors of creatinine and cystatin C equations in the selection of living kidney donors

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ABSTRACT

Background. Reliable determination of glomerular filtration rate (GFR) is crucial in the evaluation of living kidney donors. Although some guidelines recommend the use of measured GFR (mGFR), many centres still rely on estimated GFR (eGFR) obtained through equations or 24-h creatinine clearance. However, eGFR is neither accurate nor precise in reflecting real renal function. We analysed the impact of eGFR errors on evaluation and decision making regarding potential donors.

Methods. We evaluated 103 consecutive living donors who underwent mGFR via iohexol plasma clearance and eGFR by 51 creatinine- and/or cystatin C–based equations. The cut-off for living donation in our centre is GFR > 80 mL/min for donors >35 years of age or 90 mL/min for those <35 years of age. We analysed the misclassification of donors based on the cut-off for donation-based eGFR.

Results. Ninety-three subjects (90.3%) had mGFR values above (donors) and 10 [9.7% (95% confidence interval 5.4–17)] below (non-donors) the cut-off. In non-donors, most of the equations gave eGFR values above the cut-off, so donation would have

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been allowed based on eGFR. All non-donors were female with reduced weight, height and body surface. In donors, up to 32 cases showed eGFR below the cut-off, while mGFR was actually higher. Therefore an important number of donors would not have donated based on eGFR alone.

Conclusion. The misclassification of donors around the cut-off for donation is very common with eGFR, making eGFR unreliable for the evaluation of living kidney donors. Whenever possible, mGFR should be implemented in this setting.

Keywords: estimated GFR, living kidney donation, measured GFR

INTRODUCTION

Reliable evaluation of GFR is crucial to ensure acceptable renal function of both the donor and recipient after donation [1–6]. Considering renal function criteria, subjects are accepted or rejected for donation if they have GFR values higher or lower than a cut-off point [1–6]. Living kidney donation is a circumstance in which maximal accuracy of the method used to evaluate renal function is required. Thus the method used to determine GFR—either via estimation or direct measurement—is very important in the evaluation of living donors. Accordingly, some guidelines have suggested the use of measured GFR (mGFR) in living donors [1, 6]. However, this recommendation has not been implemented in many centres worldwide, which instead rely on indirect methods like 24-h creatinine clearance (CrCl) or equations based on creatinine and/or cystatin C to calculate estimated GFR (eGFR).

The error margin of eGFR in estimating real renal function is very wide, averaging ±20–30% of mGFR, as shown in several clinical conditions such as chronic kidney disease (CKD), type 2 diabetes, renal transplantation, autosomal dominant polycystic kidney disease and even in healthy subjects with normal renal function [7–16]. The variability of eGFR is such that equations can estimate GFR to be above the cut-off for donation when in reality it is lower than the threshold, and vice versa. Such errors may influence the selection of donors, resulting in clinical consequences. Kidney donation from subjects with a GFR below the cut-off may increase the risk for CKD in the donor, and contraindicating donation from subjects with acceptable values of mGFR is clearly detrimental to the recipient.

We evaluated the impact of errors in estimating GFR on decision making in living kidney donation by analysing the risk of misclassification based on the cut-off for donation.

MATERIALS AND METHODS

Evaluation of living kidney donors

Since January 2015 we have measured GFR via iohexol plasma clearance [17] for all potential living kidney donation candidates in the Canary Islands. Taking into consideration published guidelines [1–6] and the Amsterdam Forum on the Care of the Live Kidney Donor [5], in our centre the cut-off to accept donation is 80 mL/min of mGFR for donors >35 years of age and 90 mL/min for those <35 years of age. mGFR is not adjusted for body surface area (BSA). The evaluation of renal function in donors involves two steps (Figure 1). First, subjects are screened based on two consecutive reliable 24-h CrCl measurements that must be ≥80 mL/min. A 24-h CrCl <80 mL/min is only considered when eGFR [Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)] is >80 mL/min. Second, all donors undergo iohexol plasma clearance to confirm that renal function is above the cut-off for donation. When mGFR is <80 mL/min, the iohexol plasma clearance is repeated within 7–10 days to confirm the value in stable clinical conditions. Finally, several aspects are considered in the acceptance of a donor, including the value of mGFR related to the reproducibility of iohexol plasma clearance, which in our centre is 3–5%, and the age of the donor. Thus donors with an mGFR value slightly lower than the cut-off, i.e. 75 mL/min (when included in the reproducibility calculation), are frequently considered for donation if they are >50–55 years of age; donation is contraindicated in younger donors with mGFRs of similar values.

Clinical evaluation

All potential donors underwent extensive clinical evaluation to uncover conditions that would preclude donation, including laboratory analysis, renal ultrasound, computed tomography scan to evaluate renal arteries and vein anatomy and urological evaluation, among others. Weight, height, body mass index [BMI = weight (kg)/height^2 (m)] and BSA (using the equation of Du Bois and Du Bois) were determined [18]. A BMI ≥35 kg/m^2 was an absolute contraindication for donation. A standard oral glucose tolerance test was performed in all subjects to exclude diabetes and pre-diabetes. Family history of renal disease, if any, was recorded. Hypertension was defined as blood pressure ≥140/90 mmHg or the use of antihypertensive medication to lower blood pressure. Only donors with mild hypertension, that is, blood pressure that was either slightly elevated or controlled (<140/90 mmHg) with one antihypertensive agent with or without thiazide diuretics in the absence of organ lesions, were allowed to donate. Overt proteinuria or microalbuminuria (urinary excretion 30–299 mg/24 h) of any cause contraindicated donation.

mGFR with iohexol plasma clearance

On the morning of the study, 5 mL of iohexol (Omnipaque 300, GE Healthcare, Chicago, IL, USA) was injected intravenously over 2 min. Afterwards, venous (3 mL) or capillary blood (10 μL) was obtained by venous puncture or finger prick, at 120, 150, 180, 210 and 240 min [17]. In the case of finger prick blood was collected by a capillary pipette and deposited on filter paper. Iohexol was measured in plasma or dried blood spots (DBSs) as previously described [17, 19]. Both methods can be considered to be interchangeable [19]. Iohexol levels in plasma or dried blood were measured by high-performance liquid chromatography [17, 19]. Plasma iohexol clearance was calculated according to a one-compartment model and then corrected with the equation proposed by Bröchner–Mortensen [20].

eGFR

Simultaneously with iohexol plasma clearance, serum creatinine and cystatin C levels were determined to facilitate the calculation of 51 equations: 28 creatinine based, 19 cystatin C based and 4 using both markers (Supplementary data, Table S1). Although >70 equations have been described, for

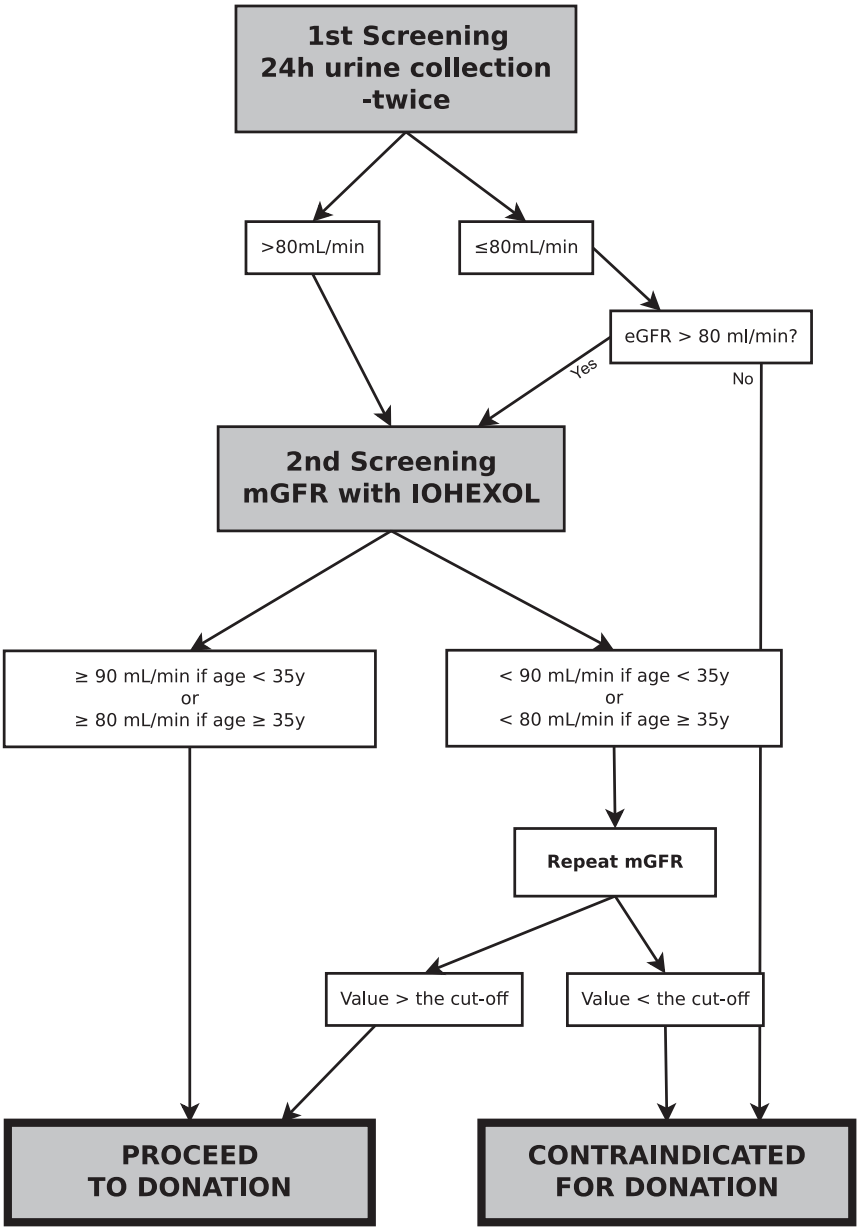

FIGURE 1: Decision tree for the evaluation of living kidney donors. Iohexol, iohexol plasma clearance; y, years.

this analysis we excluded equations developed for specific populations, such as African Americans or Asians, or diseases like diabetes or obesity. In all subjects we specifically collected weight and height at the time of mGFR determination to calculate BSA according to the equation proposed by Du Bois and Du Bois [18]. The agreement between equations and mGFR was evaluated using GFR unadjusted for BSA. When eGFR was adjusted, we reversed the adjustment of the result by applying the following equation (GFR adjusted = GFR unadjusted/BSA × 1.73).

Biochemistry

Creatinine was measured by isotope dilution mass spectrometry–traceable creatinine (cobas c711 module, Roche Diagnostics, Rotkreuz, Switzerland) and cystatin C levels by immunonephelometry (BN II System, Siemens Healthineers, Erlagen, Germany), calibrated with ERM-DA471/IFCC.

Statistical analysis

Patients were divided in two groups: those accepted (donors) or those rejected (non-donors) for donation based on mGFR. Several characteristics were compared between the groups: age, gender, height, weight, BSA, comorbidities, serum creatinine, cystatin C, 24-h CrCl and eGFR as calculated by 51 different equations based on creatinine and/or cystatin C. Analysis was performed using the Student's *t*-test, Mann–Whitney *U* test and chi-squared test as necessary. Then we evaluated the capacity of each equation to estimate GFR above or below the cut-off for donation. Finally, we performed the following analyses: positive and negative predictive values, positive and negative likelihood ratios, sensitivity, specificity, receiver operating characteristics analysis to estimate the area under the curve (AUC) and the Youden index to estimate the cut-off ratios and analyse the predictive capacity of the most common equations.

Table 1. Baseline characteristics of donors and non-donors

Characteristics	Donors	Non-donors	P-value
N	93	10	
Age (years)	48 ± 10 (23–74)	46 ± 11 (24–57)	0.60
Family history of renal disease, *n* (%)	30 (32.3)	2 (20)	0.34
Gender (female), *n* (%)	46 (52.9)	10 (100)	
Weight (kg)	75 ± 13	64.0 ± 9.5	0.009
Height (cm)	166 ± 9	158 ± 6[a]	0.008
BSA (m^2)	183 ± 19	165 ± 12[a]	0.03
BMI (kg/m^2)	27.0 ± 3.7	25.7 ± 4.0	0.33
Hypertension, yes, *n* (%)	17 (17)	1 (0)	0.24
Dyslipidaemia, yes, *n* (%)	24 (26)	3 (30)	0.54
History of renal disease, *n*	0	1[a]	
Low weight at birth, *n*	1		
Smoking, *n* (%)			
Current	33 (36)	4 (40)	0.24
Former	22 (24)	1 (10)	
eGFR, *n* (%)		2 (20)	
Serum creatinine (mg/dL)	0.86 ± 0.15	0.80 ± 0.13	0.37
Serum cystatin C (mg/L)	0.80 ± 0.12	0.82 ± 0.12	0.15
24-h CrCl (mL/min) (mL/mon)	110 ± 30	86 ± 16	0.017
MDRD (mL/min)	89 ± 17 (63.9–145.6)	78 ± 13 (47.0–95.0)	0.016
CKD-EPI$_{Cr}$ (mL/min)	97 ± 16 (68.5–140.8)	86.0 ± 15 (54.3–112.3)	0.11
mGFR (mL/min)	99.0 ± 16 (75–153)	71 ± 6 (56–81)	<0.001

[a]means $p<0.05$.
Values are presented as mean ± standard deviation (range) unless stated otherwise.

RESULTS

Donors and non-donors

A total of 103 potential donors were studied: 93 (90.3%) had mGFR values above (donors) and 10 [9.7% (95% confidence interval 5.4–17)] below (non-donors) the cut-off for donation (Table 1). Mean mGFR was lower in non-donors than in donors: 71 ± 6 versus 99.5 ± 15.5 mL/min, respectively (Table 1). All non-donors were female, with lower height, weight and BSA than donors. BSA was 10% lower in non-donors than in donors (Table 1). Age, BMI, levels of serum creatinine and cystatin C and risk factors for renal disease, including hypertension, dyslipidaemia and smoking, were comparable between groups. One donor had a previous history of reduced weight at birth, while one non-donor had an episode of acute kidney injury associated with the use of angiotensin-converting enzyme (ACE) inhibitors with a full recovery of renal function. No difference was observed regarding family history of renal disease between donors and non-donors. Also, only 2 of 10 subjects in the non-donor group were genetically related to recipients.

Predictive capacity of eGFR to classify donors and non-donors using the cut-off for donation

The negative predictive value (the capacity to classify each non-donor as such) of eGFR was low, ranging from 20 to 30% (Supplementary data, Table S2). Also, sensitivity and specificity were reduced, as was the AUC (Supplementary data, Table S2).

Renal function in subjects who would have been accepted for donation based on eGFR (non-donors based on mGFR)

Table 2 shows mGFR, 24-h CrCl and eGFR calculated by 11 equations for all subjects who would have been allowed to donate based on CrCl or an equation, whereas mGFR was below the cut-off for donation. Almost all of the equations gave eGFR values >90 or 100 mL/min. Case 1 deserves special attention since it refers to a 24-year-old woman with an mGFR of 81 mL/min. Considering her age, the cut-off for donation was >90 mL/min. In this case, some equations overestimated GFR by 40–50% [Mayo Clinic Quadratic (MCQ), CKD-EPI cystatin, (CKD-EPI$_{Cys}$) CKD-EPI creatinine-cystatin (CKD-EPI$_{Cr+Cys}$)] (Table 2). Case 2 showed eGFRs below the cut-off for all equations, whereas 24-h CrCl and the MCQ equation [21] estimated GFR to be above the cut-off. For Cases 3, 4, 8 and 9, almost all equations and CrCl estimated GFR above the cut-off for donation, in most cases yielding GFR values >90–100 mL/min. For Cases 5, 6, 7 and 10, some equations showed eGFR values above the cut-off [MCQ, Full Age Spectrum creatinine equation (FAS-cr), FAS cystatin (FAS$_{Cys}$), FAS creatinine + cystatin (FAS$_{Cr+Cys}$)], whereas others estimated GFR below the cut-off [Cockcroft-Gault (CG), abbreviated MDRD (aMDRD), CKD-EPI$_{Cr}$, Rule$_{Cy}$, CKD-EPI$_{Cy}$, CKD-EPI$_{Cr+Cys}$]. The equations failed to properly reflect similar values of mGFR. For example, seven cases showed mGFR ranging from 70 to 75 mL/min (Cases 2–4, 7–10), whereas eGFR persistently underestimated or overestimated a comparable value of mGFR, i.e. Cases 1 and 7. Also, for the same value and comparable ages (Cases 8 and 9), mGFR was 72 and the same equation showed very different results: MCQ (104 versus 85 mL/min), CKD-EPI$_{CR}$ (101 versus 86 mL/min), FAS$_{Cr}$ (106 versus 93 mL/min) and CG (101 versus 82 mL/min). Similar results were observed for the other equations (Supplementary data, Table S3). Different and even opposing values of eGFR calculated by the same equation were observed in two subjects with the same mGFR (Figure 2). For example, in different patients in whom mGFR was 70 mL/min, the CKD-EPI equation based on creatinine showed estimated values either higher (101 mL/min) or lower (61 mL/min) than real renal function (Figure 2A). Similar examples were observed for the CKD-EPI equation based on cystatin C and the MDRD and FAS equations (Figure 2A).

Renal function in subjects who would not have been accepted for donation based on eGFR (donors based on mGFR)

In donors with mGFR values above the cut-off ($n=93$), 29 (57%) of the 51 equations analysed in this study showed results below the threshold. Therefore many donors would not have been accepted for donation based on eGFR while the mGFR would have been acceptable. For creatinine-based equations, 20 (Figure 3) showed 4–32 subjects with eGFR below the cut-off (Figure 3). For example, based on the Lund–Malmö revised equation, 20 cases of 93 (21%) showed an eGFR below the cut-off for donation while the mGFR was higher than this threshold. For cystatin C–based equations, seven showed 2–10 individuals with eGFR below the cut-off. For example, the Rule equation classified 10 cases out of 93 (10%) as below the cut-off for donation while the mGFR was higher than this threshold (Figure 3). Two equations that combine creatinine and cystatin C, the Stevens and the CKD-EPI equations, showed four and three subjects below the cut-off for donation while the mGFR was higher than the threshold (Figure 3). Similar results were observed for the remaining

Table 2. eGFR and mGFR in non-donors

				Creatinine					Cystatin C				Cr + Cy	
Case	Age	mGFR	24-h CrCl	aMDRD	CKD-EPI	MCQ	CG	FAS	Le Bricon	Rule	CKD-EPI	FAS	CKD-EPI	FAS
1	24	81	109	82	96	121	103	89	129	123	128	141	126	121
2	33	71	84	52	61	83	56	62	62	48	58	66	58	64
3	35	75	104	95	112	112	119	107	101	89	108	110	109	109
4	47	73	97	79	90	90	92	93	89	79	90	95	90	94
5	49	57	60	87	100	100	107	103	86	72	82	91	89	97
6	51	65	73	73	83	103	95	89	83	67	76	87	78	88
7	51	71	74	67	75	97	81	82	103	92	101	109	87	94
8	52	72	96	89	101	104	111	106	90	75	84	95	91	100
9	57	72	83	77	86	85	82	93	93	83	89	97	88	95
10	57	70	84	66	73	89	67	81	97	86	92	101	83	90

Cr + Cy, formulas that combine creatinine and cystatin C.

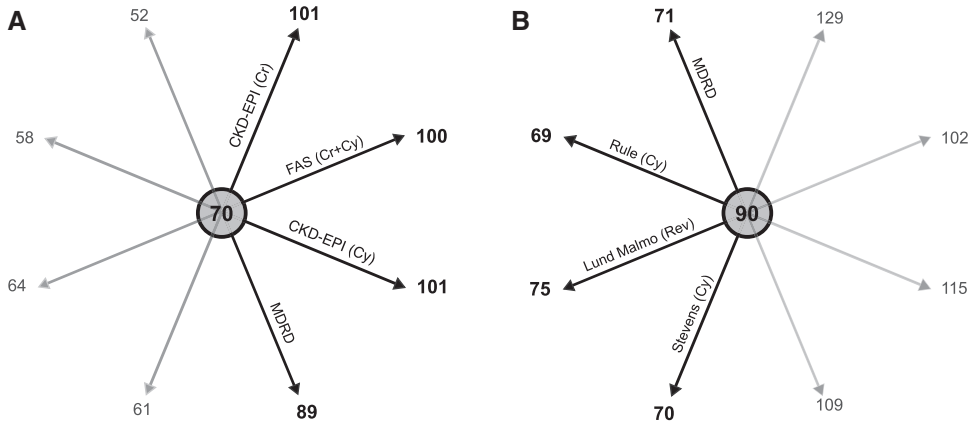

FIGURE 2: eGFR as calculated by different equations in non-donors with mGFR of 70 mL/min (range 70–73) or donors with mGFR of 90 mL/min (range 90–93). The arrows represent the estimated value calculated by the same equation in different patients with a similar GFR. Black arrows indicate over- or underestimation of real GFR, leading to incorrect acceptance or exclusion for donation in subjects with an mGFR of (**A**) ∼70 or (**B**) 90 mL/min. Equations: CKD-EPI with creatinine, CKD-EPI with cystatin C, MDRD, FAS with creatinine + cystatin C, Rule with cystatin, Lund–Malmö revised and Stevens with cystatin.

equations (Supplementary data, Table S4). Finally, as observed for the non-donors, different and even opposite values were observed in subjects with the same mGFR values of 90 mL/min, with eGFR values above or below the cut-off [109 or 71 mL/min (MDRD) and 69 or 115 mL/min (cystatin C Rule)] (Figure 2B).

DISCUSSION

Our major finding was that in the evaluation of living donors, 24-h CrCl and creatinine- or cystatin C–based equations were unreliable in classifying subjects according the cut-off for donation. In fact, for ∼10% of donors, some equations estimated GFR to be above the threshold when mGFR was actually below it, while for 10–30% of cases eGFR was below the cut-off despite mGFR levels being acceptable for donation. Thus eGFR cannot replace mGFR in the evaluation of living donors.

Misclassification around the cut-off point was particularly frequent with eGFR, reflecting erroneous over- and underestimation of real renal function. This is a consequence of the wide error range of eGFR, which varies from −30 to +30% of mGFR in 60–80% of cases and can be larger in 20–40% of cases [7–16]. Thus variations of ±10 or ±25 mL/min around any threshold are expected. This was the case for potential donors with mGFR levels around 70 or 90 mL/min, who were incorrectly classified as being above or below the cut-off for donation based on eGFR (Figure 2). Following these unreliable results would have led to the erroneous acceptance or rejection of donors.

Reliable determination of renal function is fundamental in the evaluation of living kidney donors. The cut-off point for donation has been established considering the reduction of renal function after nephrectomy and age- and gender-related normal GFR decline [21, 22]. The reduction of mGFR after nephrectomy averages 25% of pre-donation GFR, whereas mGFR decline varies from 0.4 to 1.2 mL/min/1.73 m^2/year [21, 22]. Accordingly, international guidelines recommend that living donors should have a GFR >80 mL/min before donation [1, 5]. However, this limit should be regarded with caution, as it might be more than enough for an old donor but too low for a very young one. In this sense, some guidelines propose higher cut-off values for younger donors, as they have longer life expectancy and therefore greater chance of being affected by kidney disease [1, 5]. Thus precise determination of pre-nephrectomy renal function ensures acceptable levels of GFR after donation. The misclassification of potential donors may have important consequences both for donors and recipients. Accepting donations from subjects with real GFR below and eGFR above the cut-off is clearly to the detriment of donors. On the other hand, rejecting donation based on incorrect low eGFRs is clearly detrimental to recipients.

Equations used to estimate GFR are algorithms that are primarily based on two markers, creatinine and cystatin C.

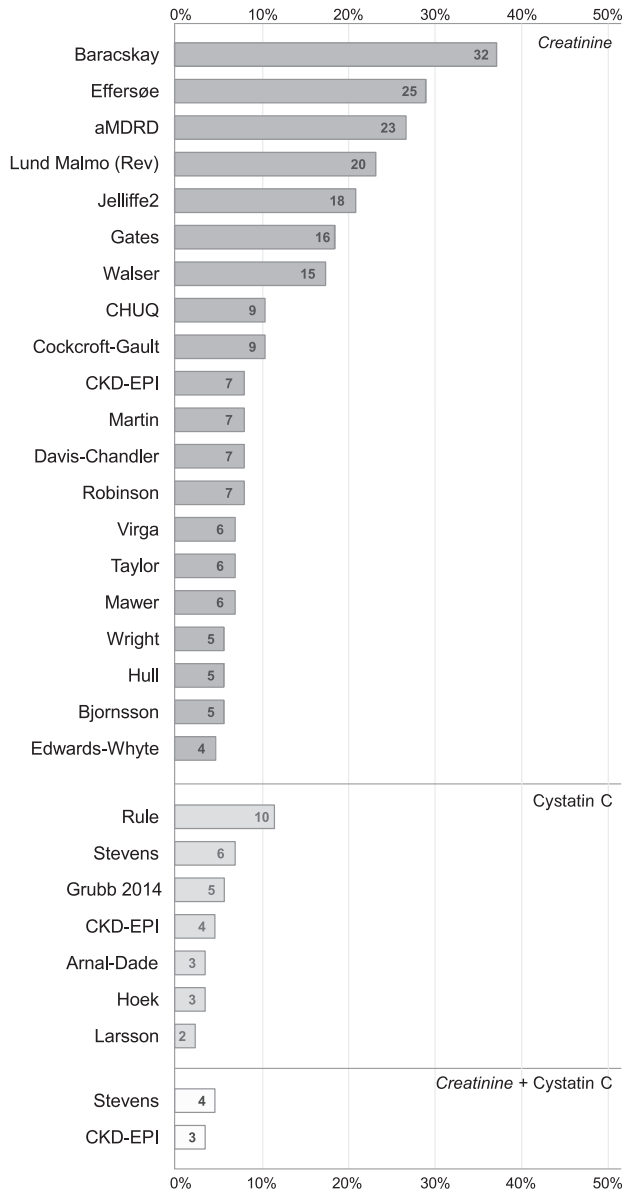

FIGURE 3: Number and percentage of donors in whom the calculated eGFR by creatinine- and/or cystatin C–based equations was below while the mGFR was actually above the cut-off for acceptance for donation. The numbers are included in each bar.

However, both markers have limitations in reflecting real GFR. Serum creatinine depends on changes in muscle mass and protein consumption, is reabsorbed and secreted by tubular cells and has a non-negligible extrarenal clearance [23–27]. Importantly, tubular creatinine excretion augments with the loss of renal function, which can explain GFR overestimation by some equations [23–27]. Cystatin C is related to obesity and inflammation and thus its levels may not always represent real renal function [28]. We evaluated a large number of equations that use creatinine or cystatin C, many of which are frequently used in clinical practice, including the MDRD, CKD-EPI and MCQ equations. Moreover, the MCQ equation was specifically developed in living donors [21]. All these equations failed to classify a number of subjects according to a cut-off for donation, showing over- or underestimation of real GFR. In 10 cases (~10% of our population), many equations and 24-h CrCl estimated GFR above the cut-off point for donation, whereas mGFR was actually lower than the cut-off. In some cases (3, 4, 5, 8 and 9; see Table 2), all equations resulted in overestimation, while in other cases (6, 7 and 10; see Table 2), diverse equations showed results above or below the threshold. Also, the error range of eGFR was unpredictable since opposite eGFR values were observed in two subjects with the same mGFR value (Figure 2): the CKD-EPI$_{Cr}$ equation estimated values either higher (101 mL/min) or lower (61 mL/min) than the cut-off when mGFR was 70 mL/min (Figure 2A). The same was observed for patients with an mGFR of 90 mL/min in whom the MDRD equation estimated values above or below the cut-off, i.e. 115 or 69 mL/min (Figure 2B). In subjects with acceptable mGFR values (donors), half of the equations underestimated renal function, leading to values below the cut-off in a number of cases (10–30%). Taken together, these results represent another example of potential errors that can occur when using eGFR to reflect real renal function.

Previous studies have evaluated the reliability of eGFR in classifying donors according to a cut-off point for donation [29, 30]. Huang *et al.* [29] designed a tool to improve the capacity of the CKD-EPI equation (creatinine- or cystatin C–based) and observed 53 and 74% true positive rates for cut-off points of 90 and 80 mL/min/1.73 m^2, respectively. This means that in one out of two (47%) or in one out of four donors (26%), these cut-offs failed to correctly estimate GFR with values of mGFR >90 or >80 mL/min/1.73 m^2, resulting a non-negligible number of potential donors not being detected. Gaillard *et al.* [30] observed that the MDRD and CKD-EPI equations had an AUC of 0.80, with a sensitivity of 1 and a specificity of 0.35 for detecting mGFR values above the cut-off for donation. This means that ~20% of true positive (donors with acceptable GFR values) and true negative cases (non-donors with reduced GFR) are undetected. Taken together, the results of these studies are in line with the low accuracy observed in our study. We think that for living donors, a higher rate of accuracy is needed to select patients before nephrectomy.

Our study reinforces the importance of mGFR in the selection of living kidney donors [31]. Gold-standard methods to evaluate GFR have been criticized as time-consuming, burdensome, difficult, impractical, cumbersome and expensive. Most of these criticisms apply to the use of inulin, which is possibly the least-used method today. The iohexol plasma clearance approach used in this study is simple, as it only requires reduced intravenous infusion of the marker (5 mL) and minimal blood sample extractions over 4 or 8 h without urine collection [32, 33]. Moreover, our group recently simplified the plasma method using the DBS technique, replacing venous blood samples with capillary blood deposited on a filter paper [17]. The DBS sampling method is very simple, uses a painless finger-prick, reduces the number of venopunctures, is safe, increases patient comfort and, importantly, shows excellent agreement with the plasma method (total deviation index = 9%) [17]. In addition, iohexol plasma clearance is not expensive, costing €100–200 [32, 33], which is negligible compared with the cost of renal transplantation. The procedure is safe, as shown in a recent publication with 2891 patients (15 147 GFR measurements), with only one treatment-related event (0.0066%) of moderate intensity [34]. Finally, as with other procedures in clinical medicine such as angiography, colonoscopy and magnetic resonance, the procedure is time-consuming; however, the benefits of accurate and precise determination of renal function in this population clearly outweigh this particular limitation.

There is no clear explanation for the reduced mGFR in non-donors (Table 2). The prevalence of risk factors for renal disease

was low and comparable for both donors and non-donors. Only one case showed a previous history of acute kidney injury with the use of ACE inhibitors, with complete recovery. Interestingly, all of the non-donors were female with reduced weight, height and BSA, characteristics that have been related to reduced nephron endowment. The number of glomeruli in normal kidneys is highly variable, ranging from 225 000 to 1 825 000 [35]. Nephron endowment is related to many factors, including reduced weight at birth, pre-term birth, short stature, low kidney mass or reduced kidney volume, among others [36–38]. Interestingly, some studies have proposed that females have fewer of glomeruli than men [39]. A recent and very important study in living kidney donors evaluated the relationship between renal histology and the number of glomeruli, as well as with total and single-nephron GFR [40]. Interestingly, female sex and shorter height were associated with a low number of nephrons and with a lower GFR. In our study, non-donors were female and of shorter height than donors, so reduced nephron number might be the cause of or a contributor to the low mGFR observed in these patients. However, we acknowledge that this study was not designed to evaluate the causes of reduced GFR in living kidney donors, which would be worth investigating in the future.

Our study has limitations and strengths. The limitations mostly pertain to the fact that our donors are of Caucasian origin and thus these results may not apply to Asians or African Americans. However, errors from calculating eGFR have been observed in these populations [41, 42]. The strengths include the use of a gold-standard method to evaluate GFR in living donors and the analysis of a large variety of equations, either creatinine- and/or cystatin C–based, to estimate renal function.

In conclusion, eGFR is not reliable in the evaluation of renal function in candidates for living kidney donation. The use of equations may lead to the acceptance of subjects with reduced real renal function in ∼10% of cases, as well as the rejection of candidates with acceptable GFR for donation in ∼10–30% of cases. Whenever possible, mGFR should be used to evaluate renal function in candidates for living kidney donation.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Lentine KL, Kasiske BL, Levey AS *et al*. KDIGO clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation* 2017; 101(8 Suppl 1): S1–S109
2. Abramowicz D, Cochat P, Claas FHJ *et al*. European renal best practice guideline on kidney donor and recipient evaluation and perioperative care. *Nephrol Dial Transplant* 2015; 30: 1790–1797
3. British Transplantation Society. *Guidelines for living kidney donor transplantation*, 4th ed. https://bts.org.uk/wp-content/uploads/2018/07/FINAL_LDKT- guidelines_June-2018.pdf
4. Gentil Govantes MÁ, Pereira Palomo PP. Assessing and selecting a live kidney donor. *Nefrologia* 2010; 30: 47–59
5. Delmonico F; Council of the Transplantation Society. A report of the Amsterdam Forum on the Care of the Live Kidney Donor: data and medical guidelines. *Transplantation* 2005; 79(6 Suppl): S53–S66
6. OPTN Policies - Organ Procurement and Transplantation Network. *Policy 14: Living donation*. http://optn.transplant.hrsa.gov/ContentDocuments/OPTN_Policies.pdf (May 2018, date last accessed)
7. Fan L, Inker LA, Rossert J *et al*. Glomerular filtration rate estimation using cystatin C alone or combined with creatinine as a confirmatory test. *Nephrol Dial Transplant* 2014; 29: 1195–1203
8. Evans M, van Stralen KJ, Schon S *et al*. Glomerular filtration rate-estimating equations for patients with advanced chronic kidney disease. *Nephrol Dial Transplant* 2013; 28: 2518–2526
9. Inker LA, Tighiouart H, Coresh J *et al*. GFR estimation using β-trace protein and β_2-microglobulin in CKD. *Am J Kidney Dis* 2016; 67: 40–48
10. Selistre L, Rabilloud M, Cochat P *et al*. Comparison of the Schwartz and CKD-EPI equations for estimating glomerular filtration rate in children, adolescents, and adults: a retrospective cross-sectional study. *PLoS Med* 2016; 13: e1001979
11. cIliadis F, Didangelos T, Ntemka A *et al*. Glomerular filtration rate estimation in patients with type 2 diabetes: creatinine- or cystatin C-based equations? *Diabetologia* 2011; 54: 2987–2994
12. Luis-Lima S, Porrini E. An overview of errors and flaws of estimated GFR versus true GFR in patients with diabetes mellitus. *Nephron* 2017; 136: 287–291
13. Luis-Lima S, Marrero-Miranda D, González-Rinne A *et al*. Estimated glomerular filtration rate in renal transplantation: the nephrologist in the mist. *Transplantation* 2015; 99: 2625–2633
14. Masson I, Maillard N, Tack I *et al*. GFR estimation using standardized cystatin C in kidney transplant recipients. *Am J Kidney Dis* 2013; 61: 279–284
15. Orskov B, Borresen ML, Feldt-Rasmussen B *et al*. Estimating glomerular filtration rate using the new CKD-EPI equation and other equations in patients with autosomal dominant polycystic kidney disease. *Am J Nephrol* 2010; 31: 53–57
16. Eriksen BO, Mathisen UD, Melsom T *et al*. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. *Kidney Int* 2010; 78: 1305–1311
17. Luis-Lima S, Gaspari F, Porrini E *et al*. Measurement of glomerular filtration rate: internal and external validations of the iohexol plasma clearance technique by HPLC. *Clin Chim Acta* 2014; 430: 84–85
18. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. *Ann Intern Med* 1916; 17: 863

19. Luis-Lima S, Gaspari F, Negrín-Mena N *et al*. Iohexol plasma clearance simplified by dried blood spot testing. *Nephrol Dial Transplant* 2017; 33: 1597–1603
20. Bröchner-Mortensen J. A simple method for the determination of glomerular filtration rate. *Scand J Clin Lab Invest* 1972; 30: 271–274
21. Rule AD, Gussak HM, Pond GR *et al*. Measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis* 2004; 43: 112–119
22. Grewal GS, Blake GM. Reference data for ^{51}Cr-EDTA measurements of the glomerular filtration rate derived from live kidney donors. *Nucl Med Commun* 2005; 26: 61–65
23. Rule AD, Larson TS, Bergstralh EJ *et al*. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004; 141: 929–937
24. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; 38: 1933–1953
25. Crim MC, Calloway DH, Margen S. Creatine metabolism in men: urinary creatine and creatinine excretions with creatine feeding. *J Nutr* 1975; 105: 428–438
26. Heymsfield SB, Arteaga C, McManus C *et al*. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983; 37: 478–494
27. Bleiler RE, Schedl HP. Creatinine excretion: variability and relationships to diet and body size. *J Lab Clin Med* 1962; 59: 945–955
28. de Vries AP, Rabelink TJ. A possible role of cystatin C in adipose tissue homeostasis may impact kidney function estimation in metabolic syndrome. *Nephrol Dial Transplant* 2013; 28: 1628–1630
29. Huang N, Foster MC, Lentine KL *et al*. Estimated GFR for living kidney donor evaluation. *Am J Transplant* 2016; 16: 171–180
30. Gaillard F, Flamant M, Lemoine S *et al*. Estimated or measured GFR in living kidney donors work-up? *Am J Transplant* 2016; 16: 3024–3032
31. Poggio E, Braun WE, Davis C. The science of stewardship: due diligence for kidney donors and kidney function in living donation—evaluation, determinants and implications for outcomes. *Clin J Am Soc Nephrol* 2009; 4: 1677–1684
32. Delanaye P, Melsom T, Ebert N *et al*. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 2: Why to measure glomerular filtration rate with iohexol? *Clin Kidney J* 2016; 9: 700–704
33. Delanaye P, Ebert N, Melsom T *et al*. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol? *Clin Kidney J* 2016; 9: 682–699
34. Gaspari F, Thakar S, Carrara F *et al*. Safety of iohexol administration to measure glomerular filtration rate in different patient populations: a 25-year experience. *Nephron* 2018; 140: 1–8
35. Rook M, Hofker HS, van Son WJ *et al*. Predictive capacity of pre-donation GFR and renal reserve capacity for donor renal function after living kidney donation. *Am J Transplant* 2006; 6: 1653–1659
36. Hughson M, Farris AB, Douglas-Denton R *et al*. Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int* 2003; 63: 2113–2122
37. Luyckx V, Brenner B. The clinical importance of nephron mass. *J Am Soc Nephrol* 2010; 21: 898–910.
38. Low Birth Rate and Nephron Number Working Group. The impact of kidney development on the life course: a consensus document for action the low birth weight and nephron number working group. *Nephron* 2017; 136: 3–49
39. Hughson MD, Douglas-Denton R, Bertram JF *et al*. Hypertension, glomerular number, and birth weight in African Americans and white subjects in the southeastern United States. *Kidney Int* 2006; 69: 671–678
40. Denic A, Mathew J, Lerman LO *et al*. Single-nephron glomerular filtration rate in healthy adults. *N Engl J Med* 2017; 376: 2349–2357
41. van Deventer HE, Paiker JE, Katz IJ *et al*. A comparison of cystatin C- and creatinine-based prediction equations for the estimation of glomerular filtration rate in black South Africans. *Nephrol Dial Transplant* 2011; 26: 1553–1558
42. Feng JF, Qiu L, Zhang L *et al*. Multicenter study of creatinine- and/or cystatin C-based equations for estimation of glomerular filtration rates in Chinese patients with chronic kidney disease. *PLoS One* 2013; 8: e57240

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