ASSESSMENT OF GFR IN THE EVALUATION OF POTENTIAL LIVING KIDNEY DONORS AT THE WITS DONALD GORDON MEDICAL CENTER (WDGMC) AND CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

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DECLARATION

I, Jacktone Odhiambo Okuthe declare that this research report is my own work. It is being submitted for the degree of Master of Medicine, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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PRESENTATIONS ARISING FROM THE STUDY

Oral Presentations:

Roche Renal and Transplant Expert Forum 1-2 April 2017

Evaluating kidney function in living donors in South Africa

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Evaluating kidney function in living donors in South Africa

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ABSTRACT

Equations that estimate GFR (eGFR) are widely used in clinical practice to estimate kidney function in sub-Saharan Africa, but have not been validated for use in this region. This study assessed the performance of eGFR equations in adults evaluated for suitability for live kidney donation against a gold standard radionuclear GFR measurement (mGFR) and determined their usefulness for screening live kidney donors in South Africa.

This study was a retrospective record review of 350 adults evaluated for living kidney donation from 1996 – 2013 at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Wits Donald Gordon Medical Centre (WDGMC). Their eGFR was calculated using CG, 4-v MDRD and CKD-EPI equations. Plasma clearance of ^{51}Cr-EDTA was used as a reference method for mGFR.

The 4-v MDRD (with and without ethnicity adjustment) and the CKD-EPI (without ethnicity adjustment) equations underestimated the mGFR (negative bias of -8 mL/min/1.73m^2, -16 mL/min/-1.73m^2 and -6.4 mL/min/1.73m^2 respectively).However, the bias associated with the average mGFR using the CG and CKD-EPI (with ethnicity adjustment) equations was not significant (2.3 mL/min/1.73m^2 and 0.6 respectively).Use of the ethnicity factor resulted in overestimation of mGFR for both the 4v-MDRD equation (by 24.2ml/min/1.73m^2 compared to 6.8 ml/min/1.73m^2 without it) and the CKD-EPI equation (by 21.8ml/min/1.73m^2, compared to 7.6ml/min/1.73m^2, without the ethnicity factor).

In conclusion, this study showed that almost half of adults screened for living donation in Johannesburg were not eligible due to comorbid hypertension, diabetes and unexplained kidney disease. In addition, the error statistics worsened as mGFR increased and all four prediction equations had a low sensitivity for determining individuals with a GFR <80 ml/min/1.73m^2. Based on the findings in this study, use of a gold standard measured GFR should be the preferred method for assessing kidney function in potential living kidney donors in South Africa.

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List of abbreviations

BMI: Body mass index

BSA: Body surface area

CCr: Creatinine clearance rate

CDC: Centre for Disease Control

CG: Cockroft-Gault

CHBAH: Chris Hani Baragwanath Academic Hospital

CI: Confidence interval

CKD: Chronic kidney disease

CKD-EPI: Chronic kidney disease-epidemiology collaboration equation without adjustment for ethnicity

CKD-EPI-e: Chronic kidney disease-epidemiology collaboration equation with adjustment for ethnicity

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CMV: Cytomegalovirus

^{51}Cr EDTA: Chromium 51 ethylene diamine tetra acetic acid

CT: Computer tomography

EBV: Epstein Barr virus

eGFR: Estimated glomerular filtration rate

ESKD: End stage kidney disease

GFR: Glomerular filtration rate

HIV: Human immunodeficiency virus

HLA: Human leucocyte antigen

IDMS: Isotope dilution mass spectrometry

IQR: Interquartile range

KDIGO: Kidney disease, improving global outcomes

LOA: Limits of agreement

MDRD: Modification of diet in renal disease

4v-MDRD: 4 variable modification of diet in renal disease equation without adjustment for ethnicity

4v-MDRD-e : 4 variable modification of diet in renal disease equation with adjustment for ethnicity

mGFR: Measured glomerular filtration rate

MRI: Magnetic resonance imaging

NHLS: National health laboratory service

NPV: Negative predictive value

P_{30} : Accuracy within 30%

PoPI: protection of personal information

PPV: Positive predictive value

REDCap: Research Electronic Data Capture

RMSE: Root mean square error

RN: Radionuclide

SAS: Statistical analysis software

Tc-99m DTPA: Technetium-99m-diethylene triamine pentaacetic acid

WDGMC: Wits Donald Gordon Medical Centre

Assessment of GFR in the evaluation of potential living kidney donors at the Wits Donald Gordon Medical Center (WDGMC) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)

1. Introduction

Chronic kidney disease (CKD) is now a major public health problem affecting an estimated 500 million people globally - that is approximately 1 in 10 adults (1, 2). There is little information on the burden of CKD in sub Saharan Africa and the mortality associated with advanced stages is high, as few people with end stage kidney disease (ESKD) can access adequate medical care (3). In countries such as South Africa where the prevalence of common risk factors such as diabetes, hypertension and HIV is high, the risk of CKD is thought to be similarly high, though there is little local data to substantiate or refute this (4). While chronic dialysis is a therapeutic option, the optimal treatment for ESKD is kidney transplantation. Potential kidney donors may be deceased or living and if living, related or unrelated to the recipient.

Living donor kidney transplants have a better outcome than deceased donor with regard to recipient and graft survival (5). In Africa, It has been reported that kidney transplantation is only done in South Africa, Nigeria, Tunisia and Egypt. The source of most of these transplants arise from living donors except in South Africa where 60 - 80% are deceased donors (4). Even in South Africa, which is regarded as a relatively wealthy country in Africa, many patients with ESKD fail to access dialysis and transplantation, particularly in the public health care sector (4). Scarce resources, shortage of donors as well as spiritual and cultural beliefs have all been said to contribute to limiting access to kidney transplantation (4, 6).

Worldwide there is an increasing trend towards the use of living donors, both related and unrelated (7). However, the transplant community in South Africa is still heavily reliant on deceased donors. According to the organ donor foundation statistics there were 231 kidney transplants in South Africa in the year 2015 and 40% were living donors (8). One of the factors that may contribute to lower than preferred living donation is the unsuitability of screened donors.

In one previous South African study, 59.7% of potential living donors were not suitable to donate for various reasons. Medical reasons for non-donation included: presence of persistent non-orthostatic proteinuria, glomerular filtration rate (GFR) below 80mL/min/1.73m^2, abnormalities of the urinary tract, uncontrolled hypertension, obesity, vasculopathy and heart disease, diabetes mellitus, infections (HIV, chronic hepatitis B and C), psychological problems, liver disease and malignancies (7). Similar findings have been documented elsewhere, such as in Italy where 56.9% of potential donors were unsuitable (9).

Kidney transplant is now established to be a safe procedure and perioperative deaths are extremely rare (4, 10). However, following unilateral nephrectomy donors are classified as having CKD due to an observed decrease in the GFR (10). In one study, both the lifespan and quality of life of carefully selected donors was found to be similar to that of the general population (11). However, a more recent study found that living kidney donors had a small absolute increase in the risk of developing ESKD over a median of 7.6 years compared to matched healthy non-donors (12). Of primary importance therefore in the screening process is an estimation of renal function to ensure that donors are not unduly compromised. Therefore, one of the most critical components in the evaluation of the living donor is the method used for assessing kidney function, usually measured by determining the glomerular filtration rate (GFR).

The GFR is the sum of filtration rates of all functioning nephrons and thus a measure of kidney function that's currently accepted as the best available measure of functioning renal mass (13). It is critical to accurately assess GFR in potential living kidney donors to ensure that the donor has sufficient functional reserve to donate one kidney without compromising their health, while the recipient receives an adequately functioning kidney. Although the GFR can be measured with precision using specific filtration markers such as inulin, 125 I-iothalamate, chromium 51 ethylene diamine tetra acetic acid (^{51}Cr EDTA), technetium-99m-diethylene triamine pentaacetic acid (Tc-99m DTPA) and iohexol, these methods are not readily available for regular clinical use. Furthermore, they are not only expensive and time consuming but also require special skills and equipment. Nevertheless, the current gold standard for direct GFR measurement is by the use of radioisotopes (^{51}Cr EDTA, Tc-99m DTPA), and iohexol (13, 14).

Renal function may also be evaluated by the use of radiologic investigations and surrogate markers. CT scanning measures functional renal volume while MRI measures renal anatomy, function and vascular morphology. Radiologic methods are similarly expensive, complex and not readily available. As an alternative, using serum creatinine as a surrogate marker for GFR, equations have been derived to estimate GFR (eGFR) including: the MDRD (modification of diet in renal disease), CKD-EPI (chronic kidney disease-epidemiology collaboration) and Cockroft-Gault equations, and 24 hour urine creatinine clearance (15, 16).

These equations have been developed in Caucasians and African Americans but are widely applied to other populations in clinical practice to estimate kidney function, including in sub Saharan Africa (17). Adjustments for ethnicity that were derived from African American studies, although widely applied to Africans, has not been validated. In fact, isolated studies have demonstrated that these adjustments for ethnicity worsen performance of the equations in SSA (14).

The Cockroft-Gault equation was derived in 1976, in Canada, using an unstandardized creatinine assay with a predominantly white male sample. The equation adjusts for weight and gender. The inclusion of the weight factor is intended to adjust for muscle mass, a determinant of serum creatinine concentration. Its performance is adversely affected in clinical situations where change in weight is not due to a change in muscle mass e.g. oedematous states and obesity. In different studies the CG has been shown to overestimate GFR by 16 to 23% (18).

The MDRD study equation was developed in the USA using data from a sample of adult patients with CKD (GFR below 60mL/min/1.73m^2), the majority of whom were Caucasian with far fewer African American participants. The 4-variable adaptation of the MDRD equation has been widely used in clinical practice since 1999 (19). It provides an estimate of GFR using serum creatinine, age, sex and an adjustment for African American ethnicity, normalized to 1.73 meter squared body surface area (BSA), the accepted average adult surface area (20). The equation should only be used when the renal function is stable and is not recommended for use in individuals with abnormal basal creatinine production caused by extreme body size/muscle mass, obesity, severe malnutrition, amputees, paraplegics or vegetarians and those on creatine supplements (16). In a study on black South African patients with CKD

at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg, the 4-variable MDRD eGFR overestimated GFR with a positive bias of 27% using the adjustment factor for African Americans, while the bias was reduced to 5% without this (14).

Because the accuracy of the 4-variable MDRD equation was poor at higher eGFR, the CKD-EPI equation was developed in 2009. The data to derive the equation were sourced from 8,254 individuals from 10 studies, including the MDRD study, and validated in an additional 16 studies containing 3,896 individuals. The variables used were the same as those for MDRD equation i.e. serum creatinine level, age, race and sex. The CKD-EPI demonstrated higher GFR levels for African Americans compared to whites at all serum creatinine levels. To accommodate this, an African American adjustment factor of 1.159 was derived, which is different from the one used in the 4-variable MDRD equation. The performance of the CKD-EPI equation is superior to the 4-variable MDRD equation at eGFR between 60 and 120 mL/min/1.73m^2, while the results are as accurate below 60 mL/min/1.73m^2 (21, 22). Again, when the adjustment factor for African ethnicity was applied to black South African patients at CHBAH, all levels of GFR were found to be overestimated but more so, for those with values below 60 mL/min/1.73m^2. Less bias was noted when the adjustment factor for African Americans was excluded (14).

Creatinine clearance over 24 hours, calculated as a rate (CCr) is an alternative method for estimating GFR. This is estimated from measuring the creatinine excreted in a 24 hour urine specimen and a serum creatinine specimen obtained during the same period (14). Fifteen percent of creatinine is actively secreted in the proximal tubule of the kidney and less in the small bowel. Both anatomical regions manifest proportionately increased secretion with worsening kidney function. As a result, the CCr overestimates glomerular filtration by about 10-40% compared to actual GFR (16). In addition, collecting urine over a 24 hour period is inconvenient to most people and therefore unlikely to be carried out with accuracy (14). For these reasons, this test is not routinely used. In a study on African Americans with hypertensive nephrosclerosis, CCr was found to weakly correlate with actual GFR (23).

All the eGFR equations have not been evaluated in Africans. In the transplant setting, there are no studies assessing the performance of eGFR in healthy donors. This is particularly relevant in South Africa because access to gold standard or measured GFR (mGFR) is limited and the cost is high, so using eGFR is cheaper, but must be scientifically justifiable. Similarly, if mGFR is recommended this needs to be substantiated by local data. Currently there are no such studies that have been published from South Africa. Guidelines for clinicians are unclear from the transplant community, and all recommendations are derived from the northern hemisphere. The 2015 clinical practice guidelines for the evaluation and follow up of live kidney donors from KDIGO recommends using eGFR derived from the 2009 CKE-EPI creatinine equation in North America, Europe and Australia. In other regions, studies are recommended to address prediction accuracy among racial and ethnic groups for whom the accuracy of eGFR is less certain (24).

2. Aim

This aim of this study was to investigate the eligibility of potential living kidney donors in the Johannesburg region who presented for investigation, to compare the performance of estimated GFR equations to a gold standard reference method for evaluating their kidney function and, in those who donated a kidney, to determine the one year recipient and graft survival after transplant.

3. Objectives

1. To describe the demographics and success rates for donation for potential living kidney donors assessed at the WDGMC and CMJAH between 1996 and 2013.
2. To determine the one year patient and graft survival in the living donor kidney transplant recipients who received a kidney from the living donors in the study sample

3. To calculate the eGFR for each potential living kidney donor using the CG, 4v-MDRD, 4v-MDRD-e, CKD-EPI, and CKD-EPI-e equations; as well as urinary CCr

4. To compare the performance of the urinary CCr, CG, 4v-MDRD, 4v-MDRD-e, CKD-EPI and the CKD-EPI-e equations to the gold standard (^{51}Cr EDTA) and determine the effect of age, weight, race and gender on their performance

5. To compare the performance of all estimating equations in relation to the measured GFR of 80ml/min/m^2-which is the mGFR level below which donors are considered ineligible for kidney donation

4. Methods

A retrospective record review of 350 adults evaluated for living kidney donation from 1996 – 2013 at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Wits Donald Gordon Medical Centre (WDGMC) was performed. Both hospitals are specialist referral sites for kidney transplantation and comprise part of the Faculty of Health Sciences Academic Teaching Hospital Complex, University of Witwatersrand, in Johannesburg, South Africa. Ethical clearance for this study was obtained from the Human Research Ethics Committee (Medical) of the University of Witwatersrand (M140922) (appendix A). Data were collected from numerous sources. Clinical data were collected from the transplant units at CMJAH and WDGMC, laboratory data were collected from NHLS, private laboratories, namely Lancet, Ampath, Wits Pathology and the radionuclear laboratory in the department of Surgery, Faculty of Health Sciences, and University of Witwatersrand.

4.1 Evaluation of potential living kidney donors

In transplant units in Johannesburg, potential donors undergo a detailed evaluation to determine their suitability. They are assessed by a senior nephrologist not involved in the care of potential recipients together with a psychologist and a social worker. Potential contraindications to donation include a history of cancer with less than 5 years of tumor-free survival, heart disease (e.g. ischaemic heart disease, valvular heart disease and cardiomyopathy), type 1 and type 2 diabetes mellitus or impaired glucose tolerance, chronic active hepatitis B or C infection, uncontrolled hypertension or hypertension with evidence of target organ damage, unexplained persistent proteinuria and hematuria, and obesity (body mass index >35 kg/m^2) (25). If none of these exclusions are found, routine medical work up is commenced with the following tests: a full blood count, urea and electrolytes, creatinine, liver function, lipid and coagulation profile, screening for the following infections - human

immunodeficiency virus (HIV), hepatitis B, C, treponema pallidum (syphilis), cytomegalovirus (CMV),Epstein Barr virus (EBV), ABO blood grouping and Centre for Disease Control (CDC) cross match, human leucocyte antigen(HLA) typing, urine for microscopy, culture and sensitivity, 24 hour urine protein excretion and / or urine protein: creatinine ratio, urine albumin creatinine ratio and a radionuclear mGFR. Other investigations required include: an electrocardiogram, a chest radiograph, abdominal ultrasound, a mammogram for women above 50 years of age and Papanicolau smears for sexually active women, prostate specific antigen levels for men over the age of 50 years. Once potential donors successfully complete the above, the last test required is a computed tomography angiogram to evaluate renovascular anatomy so that surgery can be planned (4, 26).

4.2 Data collection for potential living kidney donors

The following data were collected: age at screening (years); gender; height (cm); weight (kg); body mass index (BMI) (kg/m^2); ethnicity (self-reported as one of the following: White, Black, Coloured, Asian); hospital site for investigations (CMJAH, CHBAH, WDGMC, other) suitability for donation: if unsuitable, reason for unsuitability.

4.3 Evaluation of kidney function in potential living kidney donors

For the evaluation of kidney function, plasma clearance of ^{51}Cr-EDTA was used as the gold standard reference method for measured GFR (m-GFR) and normalised to BSA using the Du Bois equation (27).

Using serum creatinine, the following estimating equations were used to calculate eGFR:

1. *Re-expressed 4-variable Modification of Diet in Renal Disease (4-vMDRD) equation (28):*

$$eGFR\ (mL/min/1.73m^2) = 175 \times [S\text{-}Cr\ (\mu mol/L)/88.4]^{-1.154} \times age\ (years)^{-0.203} \times (0.742\ if\ female) \times (1.1212\ if\ African\ American)$$

2. *Cockroft-Gault (CG) equation (18) normalised to 1.73m^2(29):*

   eGFR (mL/min/1.73m^2) = [140-age (years) x weight (kg) x (0.85 if female) x 1.73m^2)] / [S-Cr (µmol/L) x 0.814 x BSA (m^2)]

3. *Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (30):*

   eGFR (mL/min/1.73m^2) = 141 × min (S-Cr /κ, 1)$^{\alpha}$ × max(S-Cr /κ, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 [if female] × 1.159 [if black]

   where:

   S-Cr is serum creatinine in µmol/L,

   κ is 61.9 for females and 79.6 for males,

   α is -0.329 for females and -0.411 for males,

   min indicates the minimum of S-Cr /κ or 1,

   and max indicates the maximum of S-Cr /κ or 1.

4. *24 hour urine creatinine clearance (24h CCr) in mL/min/1.73 m^2* (14)

4.4 Data collection for kidney transplant recipients

The following data were collected: date and hospital where transplanted, cold ischaemic time (minutes), HLA (human leucocyte antigen) matching based on A, B and DR antigens; delayed graft function (defined as the need for dialysis within the first 6 weeks post-transplant); surgical complications (graft nephrectomy, wound dehiscence, wound sepsis, wound hematoma or haemorrhage, sloughed ureter, urine leak, urinoma, ureteric obstruction, hydronephrosis, lymphocele, renal vein thrombosis/stenosis, renal artery thrombosis/stenosis); hospital/name of doctor where one year follow up was done; at 1 year after kidney transplant: recipient and graft survival, graft rejection (biopsy proven / or not) and serum creatinine (umol/L).

4.5 Data management

Study data were collected and managed using REDCap which is hosted at the University of Witwatersrand with assistance of the research office at the WDGMC. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an

intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources (31). It is compliant with the protection of personal information act (PoPI act), access is password protected, and has been made available to staff and students of the Faculty of Health Sciences at the University of Witwatersrand.

5. Statistical analysis

The data file was structured and cleaned in consultation with the statistician and uncorrected GFR values were deleted. Descriptive analysis of the data was carried out as follows: Categorical variables were summarised by frequency and percentage tabulation, and illustrated by means of bar charts. Continuous variables were summarised by the mean, standard deviation, median and interquartile range, and their distribution illustrated by means of histograms.

The relationship between the gold standard (mGFR) and each of the five estimates of GFR (eGFR), namely CG, *4-v MDRD and *CKD-EPI (*each with/without adjustment for African American ethnicity), was investigated using Bland-Altman plots. For the Bland-Altman analysis, mGFR was used in place of the mean of the two methods to be compared, since the mGFR is a gold standard.

The bias was regressed against the mGFR, age, gender, ethnicity, and weight to determine if these factors play a role in accounting for the observed differences between the methods.

Further statistics were derived to assess the relationship between each eGFR and mGFR in relation to the clinical criterion for eligibility for donation, which is mGFR >=80 mL/min/1.73m^2. Overall, and within each of the following mGFR subgroups: <80 and >=80 mL/min/1.73m^2 the following was determined:

- bias: median of difference between estimated and measured GFR
- % bias: median of percentage difference between estimated and measured GFR

- P_{30}: percentage of estimated GFR values within 30% of the gold standard value
- IQR: interquartile range of difference between estimated and measured GFR
- RMSE: root mean square error

The estimated GFR equations were also assessed by their sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the identification of subjects with mGFR < 80 mL/min/1.73m^2. Data analysis was carried out using SAS. The 5% significance level was used.

Calculation of sample size requirements was based on the key research question to be answered, in this case the method agreement analysis. Bland-Altman analysis requires at least 60 (preferably 100) samples (32).

6. Results

All potential donors at the WDGMC and CMJAH who were evaluated for potential living kidney donation from 1996 to 2013 were eligible. All participants had to have a serum creatinine measured using an isotope dilution mass spectrometry (IDMS) traceable assay, a gold standard measured GFR radionuclide scan (^{51}Cr EDTA) and /or 24 hour urine CCr. Those with incomplete data were excluded from the final data set.

6.1 Eligibility of potential living kidney donors

A total of 350 potential living kidney donors were reviewed, the majority of whom were evaluated at CMJAH (274/350; 78.3%). The demographics and anthropometry are depicted in table 1. The majority were obese, young women of black ethnic origin.

Table1.Characteristics of potential living kidney donors at CMJAH and WDGMC (1996-2013)

Variable	Category	Overall
		n (%)
Age	37.0 years (sd 8.9; range 19-59 years)	
Weight	71.5 kg (sd 13.4; range 43-116 kg)	
Height	166.5 cm (sd 9.6; range 144-195 cm)	
Body mass index (BMI)	26.2 kg/m^2 (sd 4.0; range 16.6-36.4 kg/m^2)	
Hospital	CMJAH	274/350 (78.3%)
	WDGMC	68/350 (19.4%)
	Other referral sites	8/350 (2.3%)
Gender	female	206/350 (58.9%)
Ethnicity (self-reported)	black	139/350 (39.7%)
	white	115/350 (32.9%)
	asian/indian	27/350 (7.7%)
	mixed race	23/350 (6.6%)

Of those evaluated, 52.3% were suitable for donation. The main reasons for unsuitability were obesity (31/166; 18.7%), hypertension (26/166; 15.7%) and unexplained kidney disease (22/166; 13.3%) as illustrated in table 2.

Table 2: Potential living kidney donor suitability for donation at CMJAH and WDGMC (1996-2013)

Variable	Category	Overall
		n (%)
Kidney function predonation [median(IQR);range]	mGFR	93.3 ml/min/1.73m^2 (82.3-105.8);36.1-150.7
	4-vMDRD	77.6 ml/min/1.73m^2 (67.8-92.0);43.6-138.5
	4-vMDRD-e	84.7 ml/min/1.73m^2 (71.7-102.8);43.6-167.9
	CG	95.2 ml/min/1.73m^2 (83.7-110.7);51.7-162.7
	CKD-EPI	95.0 ml/min/1.73m^2 (80.5-113.2);49.9-152.0
Suitable for kidney donation	Yes	183/350 (52.3%)
	No	166/350 (47.4%)
Reason for donor unsuitability (n=166)	**Donor**	
	obesity	31/166 (18.7%)
	hypertension	26/166 (15.7%)
	low GFR(GFR<80 mL/min/1.73m^2) and unexplained proteinuria/haematuria	22/166 (13.3%)
	abnormality of urinary tract	21 /166 (12.7%)
	psychological / social problems	20/166 (12.0%)
	positive crossmatch / ABO incompatibility	14/166 (8.4%)
	HIV infection	8/166 (4.8%)
	chronic hepatitis B or C infection	8/166 (4.8%)
	tuberculosis	1/166 (0.6%)
	autoimmune disease (thyroid/sarcoidosis)	5/166 (3.0%)
	unexplained anaemia	3/166 (1.8%)
	diabetes	2/166 (1.2%)
	ischaemic heart disease/aortic aneurysm	2/166 (1.2%)
	malignancy	1/166 (0.6%)
	liver disease	1/166 (0.6%)
	Recipient	
	recipient too ill / died	10/166 (6.0%)
	transferred to another hospital	2/166 (1.2%)

6.2 Characteristics of living donor kidney transplant recipients

There was a gradual increase in transplantation rates from 1996 to 2003, with a period of decline after 2004 and a subsequent surge after 2011 (Figure 1). The majority of transplants were carried out at CMJAH (114/183; 62.3%).

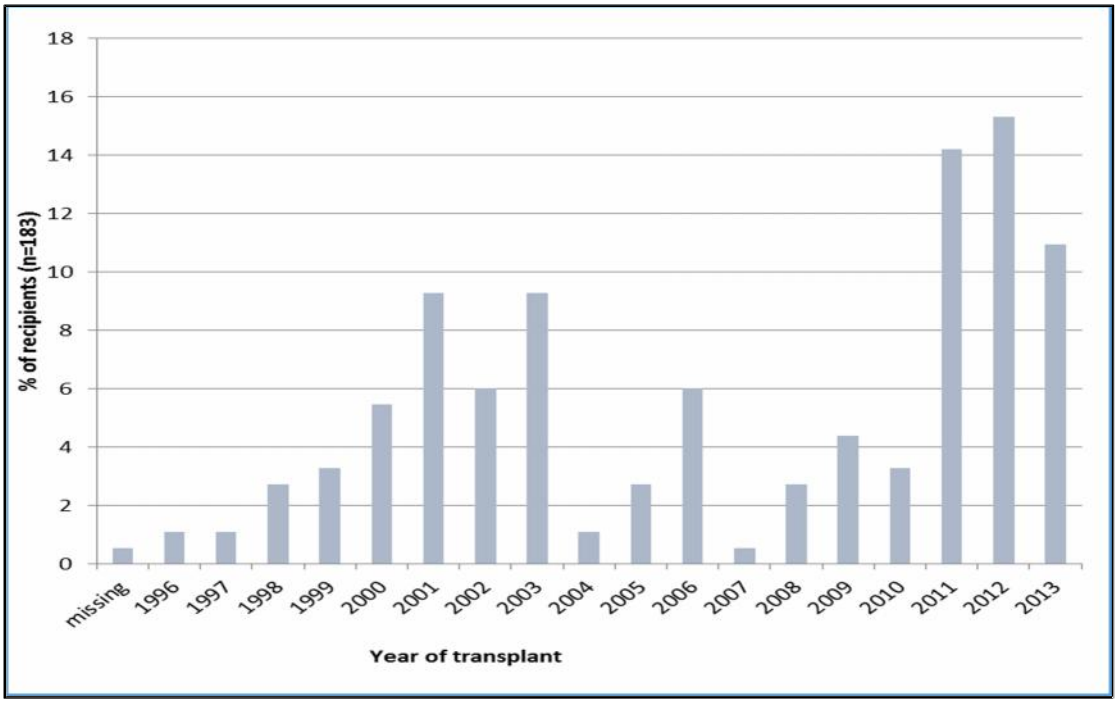

Figure 1. Living donor kidney transplant rates at CMJAH and WDGMC between 1996 and 2013

There were very few surgical complications observed in the recipients post-transplant (13/165; 7.1%), with recipient and graft survival rates at 1 year of 86.3% and 82.5% respectively. For those whose grafts survived to 1 year, the median serum creatinine was 100 µmol/l (IQR 82-143; range 23-733 µmol/l) (Table 3).

Table 3. Outcomes for living donor kidney transplant recipients at CMJAH and WDGMC between 1996 and 2013

		n (%) Recipients
Hospital	CMAJH	114/183 (62.3%)
	WDGMC	69/183 (37.7%)
HLA match	0	10/183 (5.5%)
	1	23/183 (12.6%)
	2	19/183 (10.4%)
	3	54/183 (29.5%)
	4	35/183 (19.1%)
	5	4/183 (2.2%)
	6	18/183 (9.8%)
	missing	20/183 (10.9%)
Delayed graft function	no	146/183 (79.8%)
	yes	4/183 (2.2%)
	missing	33/183 (18.0%)
Surgical complications	no	133/183 (72.7%)
	yes	13/183 (7.1%)
	missing	37/183 (20.2%)
Surgical complications: type (n=13)	graft nephrectomy	6/13 (46.2%)
	ureteric	5/13 (38.5%)
	wound hematoma / haemorrhage	2/13 (15.4%)
	renal artery thrombosis/stenosis	2/13 (15.4%)
	renal vein thrombosis/stenosis	1/13 (7.7%)
	wound dehiscence/sepsis	0/13 (0.0%)
	lymphocele	0/13 (0.0%)
	other	12/13 (92.3%)
Graft rejection in first 12 months	no	95/183 (51.9%)
	yes	14/183 (7.7%)
	missing	74/183 (40.4%)
Diagnosis of rejection (n=14)	clinical	8/14 (57.1%)
	biopsy proven acute rejection	6/14 (42.9%)
	missing	1/14 (7.1%)
Recipient alive at 1year	yes	158/183 (86.3%)
	no	2/183 (1.1%)
	missing	23/183 (12.6%)
Graft survived at 1year	yes	151/183 (82.5%)
	no	13/183 (7.1%)
	missing	19/183 (10.4%)

6.3 Performance of eGFR equations in relation to mGFR (^{51}Cr EDTA)

An assessment was done to determine the amount of data available for Bland-Altman comparisons prior to comparing the performance of eGFR equations in relation to mGFR. Of a total sample of 350 potential donors, the main method comparisons are limited to 154-233 cases, i.e. 44-66% of the data set (appendix C). There was an insufficient sample size for CCr and it was therefore excluded from the analysis. The median donor mGFR, for all those who screened, was 93.3 mL/min/1.73m^2 (IQR 82.3-105.8 mL/min/1.73m^2; range 36.1-150.7 mL/min/1.73m^2). Details of Bland-Altman analyses for each of the eGFR equations will follow, namely the CG equation, 4v-MDRD (without adjustment for ethnicity); 4v-MDRD-e (with adjustment for ethnicity); CKD-EPI (without adjustment for ethnicity); CKD-EPI-e (with adjustment for ethnicity). For all Bland Altman analyses the difference (bias) was calculated as [eGFR – mGFR]. In all the plots that are depicted: black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA).

6.3.1. Comparison of mGFR to the CG equation

There was no significant bias between the average GFR predicted by the CG equation and mGFR. The CG equation yielded an average GFR that was 2.3 mL/min/1.73m^2 higher than the mGFR. Although the average bias between the two methods was not significant, the bias became more negative (-0.54 mL/min/1.73m^2) for every unit increase in mGFR. This was confirmed by a regression of the bias vs mGFR, which showed that the bias was significantly affected by mGFR ($p<0.0001$), but there was no significant difference in the variation of the bias with respect to mGFR. The adjusted LOA are shown by the red lines in the Bland-Altman plot in figure 2.

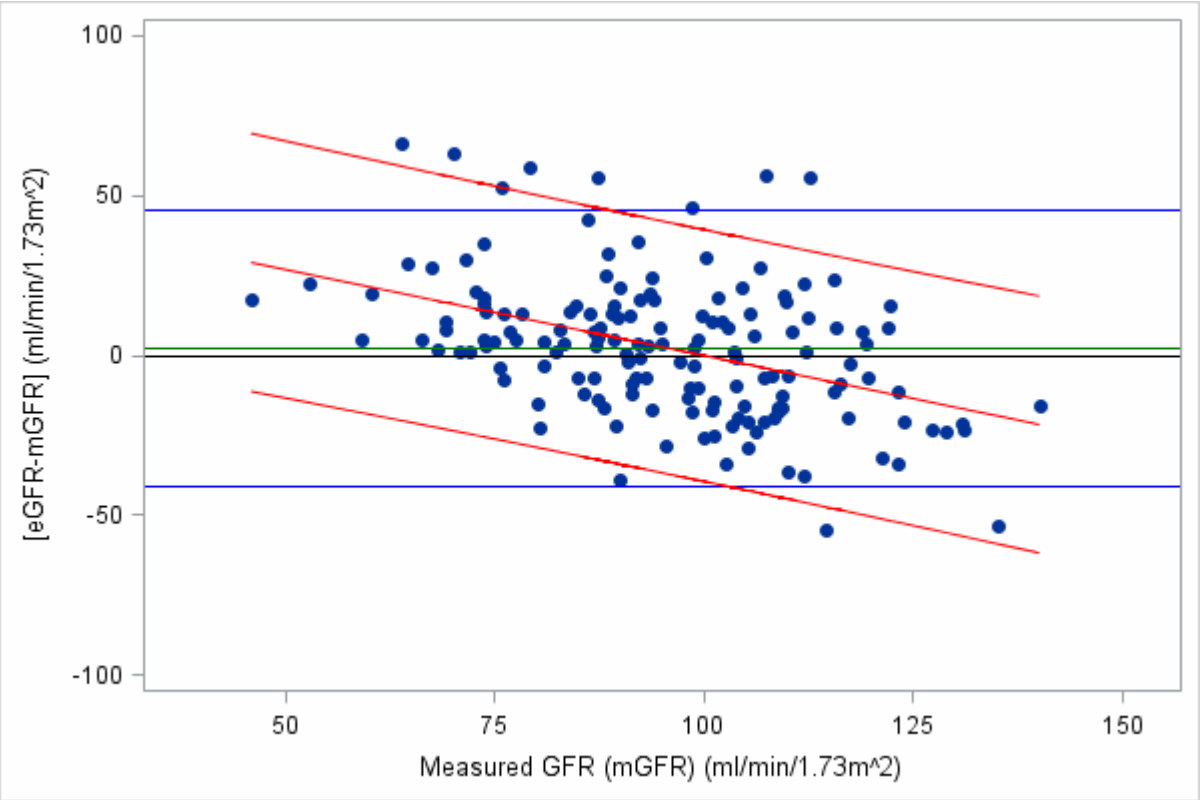

Figure 2. Bland-Altman plot for CG equation vs mGFR
(black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA); red lines: adjusted 95% limits of agreement (LOA))

Regression of the CG measurements on the mGFR measurements showed poor correspondence. By adding further terms to the regression between the bias (dependent variable) and mGFR (independent variable), the effect of age, gender, ethnicity and weight on the bias was explored. There was no effect of ethnicity ($p=0.079$) or weight ($p=0.30$) but there were significant effects of the remaining variables on the bias which included: a negative bias of -0.80 mL/min/1.73m^2 for every year of age ($p<0.0001$) and a positive bias of +7.0 mL/min/1.73m^2 for females compared to males ($p=0.042$).

When age and gender, as well as their interaction, were included in the regression the interaction term was not significant and was removed. The main effects of age and gender persisted and were significant ($p<0.0001$, and 0.029 respectively) with a negative bias (-0.80 mL/min/1.73m^2) for every year of age, and a positive bias (+7.0 mL/min/1.73m^2) for females compared to males. These effects are illustrated in table 4.

Table 4: Regression of CG on mGFR

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	88.30	12.03	7.34	<.0001
mGFR	-0.64	0.09	-7.28	<.0001
age at screening	-0.80	0.18	-4.55	<.0001
gender female	7.03	3.18	2.21	0.029

6.3.2 Comparison of mGFR to 4v-MDRD equation

There was a significant negative bias between these two methods. On average, the 4v-MDRD was 16 mL/min/1.73m^2 lower than mGFR. However, this bias was not constant across all levels of mGFR, rather it became increasingly negative (-0.77 mL/min/1.73m^2) for every unit increase in mGFR. This effect was confirmed by a regression of the bias vs mGFR, which showed that the bias was significantly affected by mGFR (p<0.0001) but there was no significant difference in the variation of the bias with respect to mGFR. The red lines in figure 3 show the adjusted LOA.

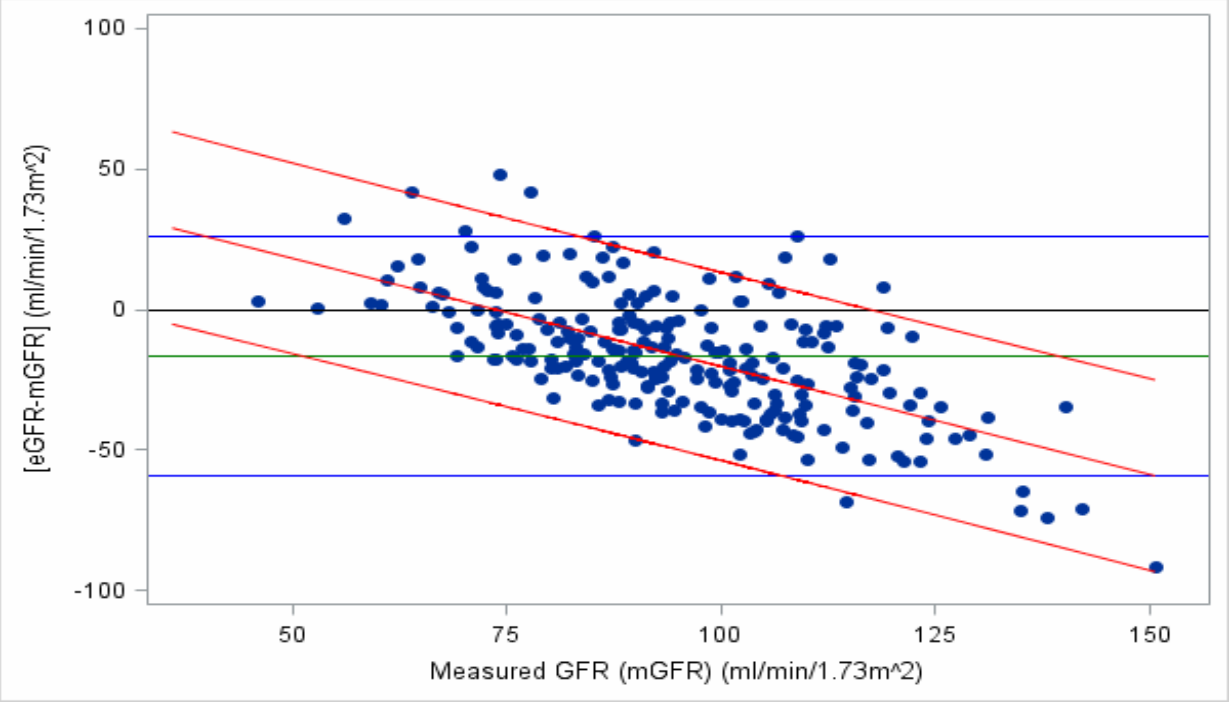

Figure 3. Bland-Altman plot for 4v-MDRD vs mGFR
(black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA); red lines: adjusted 95% limits of agreement (LOA))

Regression of the 4v-MDRD measurements on the mGFR measurements showed their correspondence was poor. By adding further terms to the regression between the bias and mGFR, the effect of age, gender, ethnicity and weight on the bias was explored. There was no effect of gender (p=0.12) but there were significant effects of the remaining variables on the bias which included: a negative bias of -0.41 mL/min/1.73m^2 for every year of age (p=0.0014); a negative bias of -0.32 mL/min/1.73m^2 for every kilogram of weight (p=0.0006); in black donors compared to white, a positive bias of 6.8 mL/min/1.73m^2 (p=0.013) was shown.

When age, ethnicity and weight, as well as their interactions, were included in the regression, the interaction terms and the effect of ethnicity were not significant and were removed. However, the main effects of age and weight remained significant (p=0.037 and 0.003 respectively) with a negative bias for every year of age (-0.30 mL/min/1.73m^2) and every kilogram of weight (-0.28 mL/min/1.73m^2). These effects are demonstrated in table 5.

Table 5: Regression of 4v-MDRD on mGFR

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	81.00	10.68	7.58	<.0001
mGFR	-0.68	0.07	-9.26	<.0001
age at screening	-0.30	0.14	-2.10	0.037
weight	-0.28	0.09	-2.99	0.0031

6.3.3. Comparison of mGFR to 4v-MDRD-e equation

There was a significant negative bias between these two methods. The average the 4v-MDRD-e was 8 mL/min/1.73m^2 lower than mGFR. However, this bias was not constant across all levels of mGFR, rather it became increasingly negative (-0.70 mL/min/1.73m^2) for every unit increase in mGFR. This effect was confirmed by a regression of the bias vs mGFR, which showed that the bias was significantly affected by mGFR (p<0.0001), but there was no significant difference in the variation of the bias with respect to mGFR. The adjusted LOA are shown by the red lines in the Bland-Altman graph in figure 4.

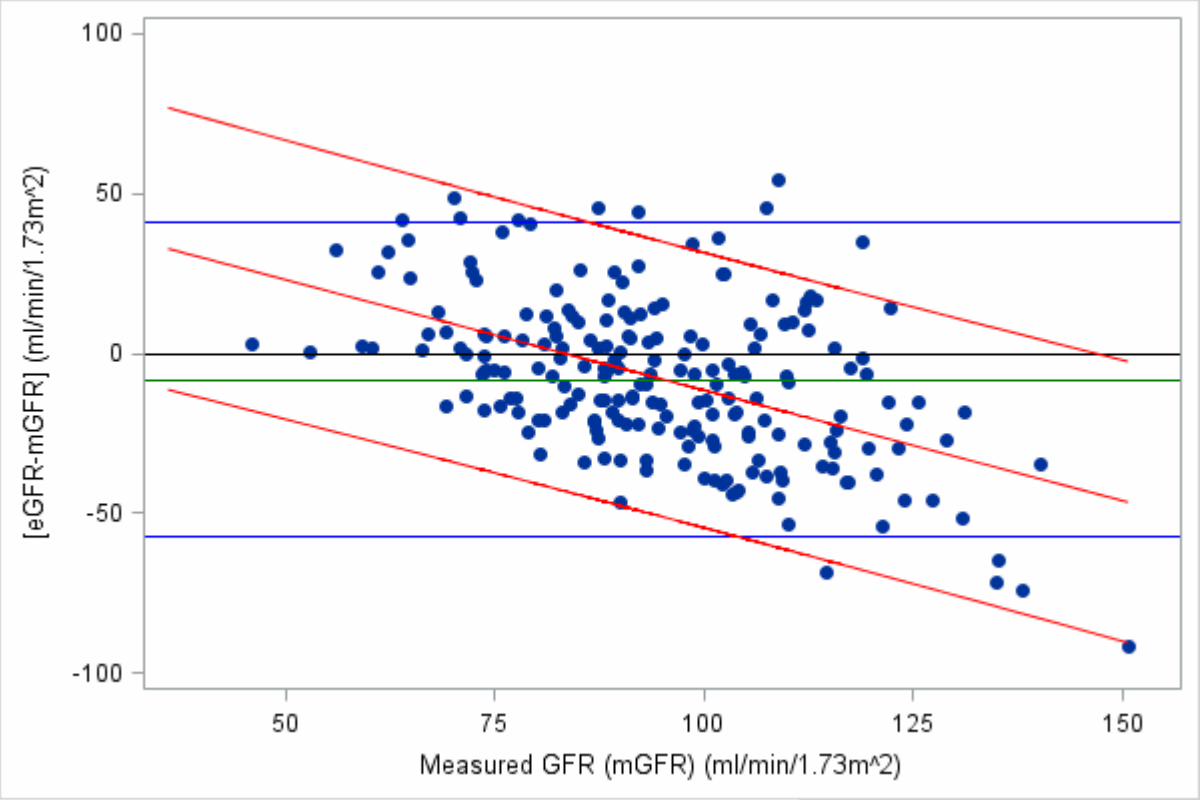

Figure 4. Bland-Altman plot for 4v-MDRD-e vs mGFR
(black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA); red lines: adjusted 95% limits of agreement (LOA))

Regression of the 4v-MDRD-e measurements on the mGFR measurements revealed poor correspondence. By adding further terms to the regression between the bias and mGFR, the effect of age, gender, ethnicity and weight on the bias was explored. There was no effect of gender (p=0.73) but there were significant effects of the remaining variables on the bias which included: a negative bias of -0.58 mL/min/1.73m^2 for every year of age (p=0.0009); a negative bias of -0.47 mL/min/1.73m^2 for every kilogram of weight (p=0.0001); in black patients compared to white, a positive bias of 24.2mL/min/1.73m^2 (p<0.0001) was demonstrated.

When age, ethnicity and weight, as well as their interactions, were included in the regression the interaction terms were not significant and were removed. However, the main effects of age, ethnicity and weight remained significant (p=0.026, <0.0001, and 0.014 respectively) with a negative bias for every year of age (-0.36 mL/min/1.73m^2) and kilogram of weight (-0.26 mL/min/1.73m^2) and a positive bias (+21.7 mL/min/1.73m^2) for Black compared to white donors. These effects are demonstrated in table 6.

Table 6: Regression of 4v-MDRD-e on mGFR

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	73.17	12.78	5.73	<.0001
mGFR	-0.60	0.08	-7.35	<.0001
age at screening	-0.36	0.16	-2.25	0.026
race asian	5.33	5.15	1.04	0.30
race black	21.67	3.19	6.79	<.0001
race coloured	-6.36	4.81	-1.32	0.19
race white	0.00	.	.	.
weight	-0.26	0.11	-2.49	0.014

6.3.4. Comparison of mGFR to the CKD-EPI equation

There was a significant negative bias between the two methods. On average the CKD-EPI method underestimated GFR by 6.4 mL/min/1.73m^2. However, the bias was not constant across all levels of mGFR, rather it became increasingly negative (-0.74 mL/min/1.73m^2) for every unit increase in mGFR. This effect was confirmed by a regression of the bias vs mGFR, which showed that the bias was significantly affected by mGFR ($p<0.0001$), but there was no significant difference in the variation of the bias with respect to mGFR. The bias became more negative by an estimated 0.74 mL/min/1.73m^2 for every unit increase in mGFR. The adjusted LOA are shown by the red lines in the Bland-Altman plot (figure 5).

Regression of the CKD-EPI measurements on the mGFR measurements revealed their correspondence was poor. By adding further terms to the regression between the bias and mGFR, the effect of age, gender, ethnicity and weight on the bias was explored. There was no effect of gender ($p=0.40$) but there were significant effects of the remaining variables on the bias which included: A negative bias of -0.64 mL/min/1.73m^2 for every year of age ($p<0.0001$); a negative bias of -0.35 mL/min/1.73m^2 for every kilogram of weight ($p=0.0001$); in black donors compared to white, a positive bias of 7.6 mL/min/1.73m^2 ($p=0.0046$) was demonstrated.

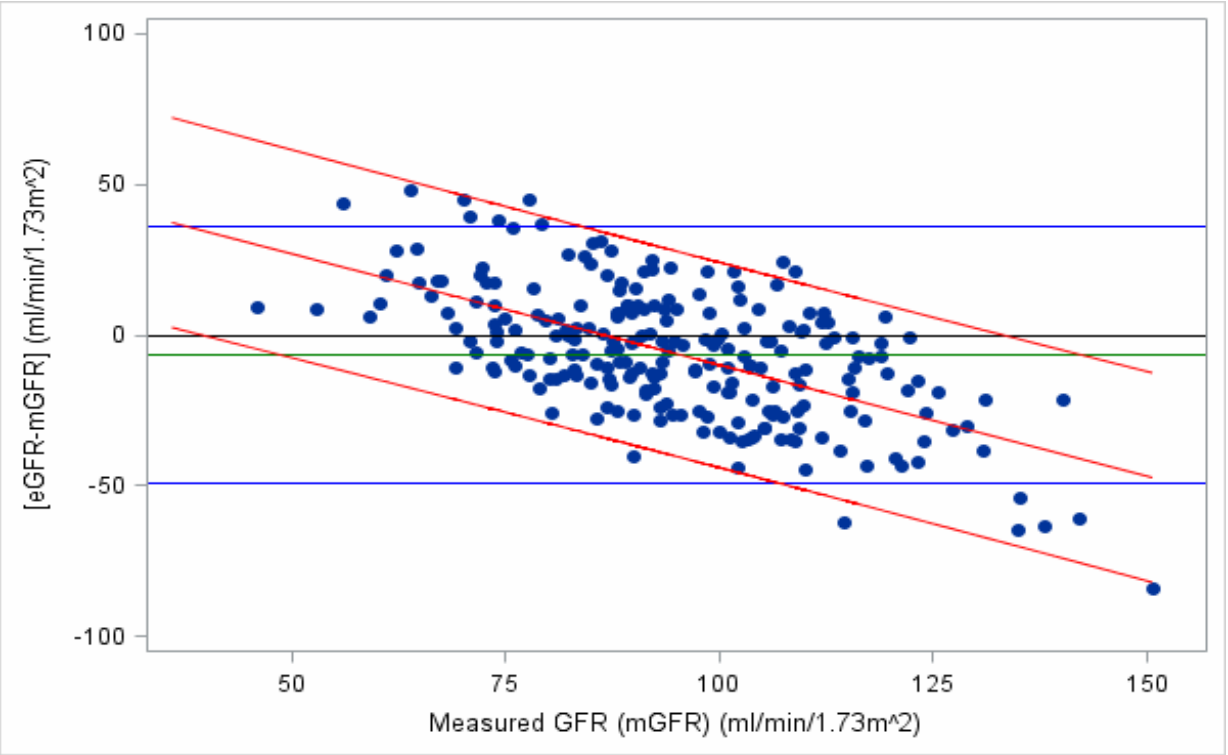

Figure 5. Bland-Altman plot for CKD-EPI equation vs mGFR
(black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA); red lines: adjusted 95% limits of agreement (LOA))

When age, ethnicity and weight, as well as their interactions, were included in the regression, the interaction terms were not significant and were removed. However, the main effects of age and weight remained significant ($p<0.0001$ and 0.0040 respectively) with a negative bias for every year of age (-0.57 mL/min/1.73m^2) and kilogram of weight (-0.26 mL/min/1.73m^2). Post-hoc tests showed no ethnicity differences. These effects are depicted in table 7.

Table 7: Regression of CKD-EPI on mGFR

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	92.57	10.91	8.48	<.0001
bamean	-0.62	0.07	-8.91	<.0001
age at screening	-0.57	0.14	-4.14	<.0001
race asian	5.47	4.39	1.25	0.21
race black	4.07	2.73	1.49	0.14
race coloured	-6.83	4.10	-1.67	0.097
race white				
weight	-0.26	0.09	-2.92	0.004

6.3.5. Comparison of mGFR to the CKD-EPI-e equation

There was no significant bias between the average GFR values obtained by the CKD-EPI-e and the mGFR; although the average GFR obtained by the CKD-EPI (with adjustment for ethnicity) was 0.6 mL/min/1.73m^2 higher than the mGFR, this was not significantly different to zero. However, the bias was not constant across all levels of mGFR, rather it became increasingly negative (-0.67 mL/min/1.73m^2) for every unit increase in mGFR. Thus the estimating equation progressively underestimated GFR with preserved kidney function. This effect was confirmed by a regression of the bias vs mGFR, which showed that the bias was significantly affected by mGFR ($p<0.0001$), but there was no significant difference in the variation of the bias with respect to mGFR. The bias became more negative by an estimated 0.67 mL/min/1.73m^2 for every unit increase in mGFR. The red lines in the Bland-Altman plot (figure 6) show the adjusted LOA

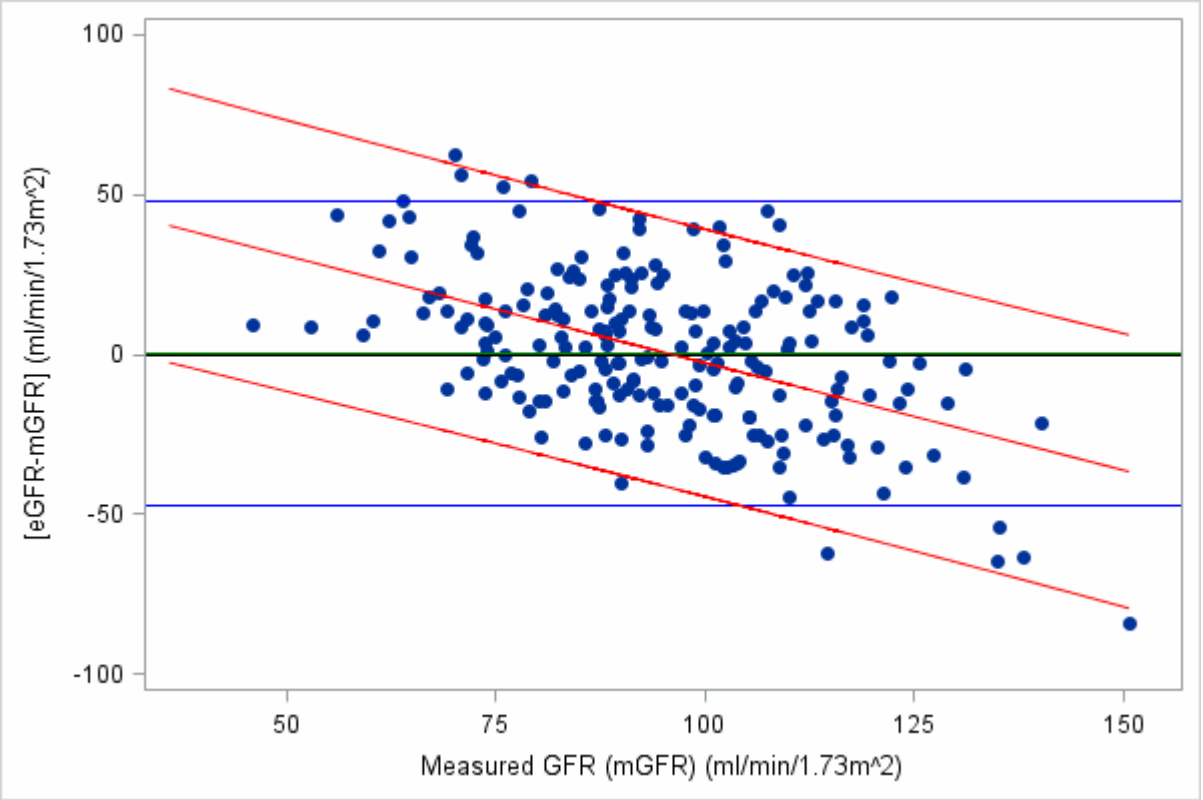

Figure 6. Bland-Altman plot for CKD-EPI-e vs mGFR
(black line = reference line for zero bias; green line = mean bias; blue lines: 95% limits of agreement (LOA); red lines: adjusted 95% limits of agreement (LOA))

Regression of the CKD-EPI- e measurements on the mGFR measurements showed that their correspondence was poor. By adding further terms to the regression between the bias and mGFR, the effect of age, gender, ethnicity and weight on the bias was explored. There was no effect of gender (p=0.98) but there were significant effects of the remaining variables on the bias which included: a negative bias of -0.82 mL/min/1.73m^2 for every year of age (p<0.0001); a negative bias of -0.49 mL/min/1.73m^2 for every kilogram of weight; in black donors compared to white, a positive bias of 21.8 mL/min/1.73m^2 (p<0.0001) was observed.

When age, ethnicity and weight, as well as their interactions, were included in the regression the interaction terms were not significant and were removed. However, the main effects of age, ethnicity and weight remained significant (p<0.0001, <0.0001, and 0.0045 respectively) with a negative bias for every year of age (-0.61 mL/min/1.73m^2) and kilogram of weight (-0.28 mL/min/1.73m^2) and a positive bias (+18.2 mL/min/1.73m^2) for Black compared to White donors. These effects are demonstrated in table 8.

Table 8: Regression of CKD-EPI-e on GFR

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	92.91	11.69	7.95	<.0001
mGFR	-0.60	0.08	-7.97	<.0001
age at screening	-0.61	0.15	-4.15	<.0001
race asian	5.38	4.70	1.14	0.25
race black	18.20	2.93	6.21	<.0001
race coloured	-6.88	4.39	-1.57	0.12
race white	0.00	.	.	.
weight	-0.28	0.10	-2.88	0.0045

6.4 Performance of eGFR equations with reference to the clinical cut-off of mGFR >=80 mL/min/1.73m^2

When assessing kidney function in donors, a clinical cut-off of mGFR >=80 mL/min/1.73m^2 defines eligibility for kidney donation, across all age groups. Based upon this practice, the performance of the eGFR equations that were evaluated in this study were further assessed. The dataset was divided into two groups: GFR <80 mL/min/1.73m^2 and >=80 mL/min/1.73m^2. For each group the following parameters were calculated: median percentage bias (difference between eGFR and mGFR); 95% confidence interval for the percentage bias; median bias (difference between eGFR and mGFR); IQR for the bias; P30 – which is the accuracy within 30% of measured GFR, and root mean squared error (table 9).

Table 9. Performance of eGFR equations with reference to the clinical cut-off of mGFR >=80 mL/min/1.73m^2 for eligibility for kidney donation

Equation	mGFR (mL/min / 1.73m^2)	n	Median percentage bias (%)	95% CI for percentage bias (%)	P_{30} (%)	Median Bias (mL/min/ 1.73m^2)	Bias (IQR) (mL/min/ 1.73m^2)	RMSE (mL/min/ 1.73m^2)
4v-MDRD	<80	46	1.5	-7.2 to 8.8	84.8	0.9	-8.9 to 10.1	17.1
	>=80	187	-22.3	-24.7 to -19.0	69.0	-20.9	-34.6 to -7.7	29.4
	Overall	233	-18.6	-22.2 to -15.9	72.1	-17.0	-30.8 to -4.5	27.4
4v-MDRD-e	<80	43	6.0	1.1 to 19.2	65.1	4.2	-5.6 to 25.2	21.7
	>=80	170	-13.9	-17.8 to -6.6	74.1	-14.2	-28.4 to 4.1	27.3
	Overall	213	-7.4	-14.2 to -5.0	72.3	-6.7	-24.3 to 6.0	26.3
CG	<80	31	17.1	6.8 to 37.2	64.5	13.0	4.5 to 27.1	26.2
	>=80	123	-2.2	-7.8 to 3.8	88.6	-2.1	-16.5 to 11.7	20.8
	Overall	154	3.4	-1.1 to 6.6	83.8	3.1	-13.5 to 13.5	22.0
CKD-EP	<80	46	15.8	5.6 to 26.5	73.9	9.6	-1.9 to 20.0	21.1
	>=80	187	-10.5	-13.8 to -5.9	82.4	-10.8	-25.5 to 2.0	22.9
	Overall	233	-6.4	-10.4 to -2.3	80.7	6.0	-19.2 to 7.4	22.6
CKD-EPI-e	<80	43	19.0	12.4 to 28.1	67.4	12.6	0.7 to 34.0	27.2
	>=80	170	-2.7	-8.3 to 2.3	81.8	-2.5	-19.1 to 13.3	23.5
	Overall	213	2.0	-2.8 to 7.1	78.9	1.9	-14.8 to 15.4	24.3

In order to determine whether the eGFR equations could assist clinicians to correctly identify potential donors for donation, the PPV and NPV to determine donors with GFR<80 mL/min/1.73m^2 was calculated for each of the estimation equations (table 10).

Table 10. Statistics to identify donors with GFR < 80 mL/min/1.73m^2

Equation	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
MDRD	233	74 (59-86)	43 (36-51)	24 (17-32)	87 (79-93)
MDRD-e	213	58 (42-73)	61 (53-68)	27 (18-37)	85 (78-91)
CG	154	48 (30-67)	84 (76-90)	43 (26-61)	87 (79-92)
CKD-EPI	233	48 (33-63)	67 (60-74)	27 (17-37)	84 (77-89)
CKD-EPI-e	213	42 (27-58)	77 (70-83)	32 (20-45)	84 (77-89)

7. Discussion

This is one of the few studies from the sub Saharan African region which has evaluated the performance of various GFR estimation equations in relation to a gold standard measured GFR method. In addition, it is the first to assess the performance of these estimating equations in potential kidney donors with predominantly normal kidney function (median measured GFR 93.3 mL/min/1.73m^2 (IQR 82.3-105.8 mL/min/1.73m^2; range 36.1-150.7 mL/min/1.73m^2).

The majority (39.7%) of potential donors in this study were young women of black ethnic origin (table 1). These findings are similar to those of a previous South African study where the mean donor age was 35.2 years, although only 24% were black (7). This study is therefore particularly relevant because the young kidney donor population has a predictably long life expectancy which may be compromised if their kidney function is not adequately evaluated at donation. Furthermore, the donor work up can be more costly due to measured GFR testing (when compared to a serum creatinine and estimated GFR calculation) and its use requires scientific justification. This cost may be compounded by the high failure rates of screened donors, as demonstrated in this study, which necessitates screening of several donors per recipient. Therefore, an accurate and affordable eGFR equation for assessing kidney function in healthy donors would be ideal, particularly in resource constrained environments.

Almost half of the potential donors screened were found to be unsuitable for living kidney donation (47.7%) (table 2). These results concur with a previous study from

South Africa and with internationally published data. In a study done at CMJAH in Johannesburg, South Africa, it was found that 59.7% of potential living kidney donors either withdrew or were withdrawn due to medical and non-medical reasons (7). Similarly, internationally, in a study done at St. Michael's hospital in Canada to determine the causes of living kidney donor rejection and deferral, 50.2% of potential living kidney donors were rejected due to medical reasons (5). In a Catholic university in Rome, Italy, a study was done to determine reasons for exclusion of potential living kidney donors referred to the centre and 56.9% of potential living kidney donors were excluded due to medical and non-medical reasons (9).

With regard to the reasons for unsuitability for live kidney donation, in this study the main reasons were obesity, hypertension, abnormalities of the urinary tract and unexplained glomerular disease, characterised by a low GFR (GFR< 80 mL/min/1.73m^2), and or unexplained haematuria/proteinuria (table 2). This is similar to other studies where it was found that the main reasons for non-donation were renal in origin (including non-orthostatic proteinuria, abnormal GFR and urological problems), obesity, hypertension and diabetes (5, 7, 9). This finding suggests that there is a high burden of undiagnosed CKD in this community. Of those who were found to be eligible, all underwent living kidney donation. With regard to the outcomes of these procedures, there were very few surgical complications in the recipients, and no complications were documented in the donors. Recipient and graft survival at one year post transplant was 86.3% and 82.5% respectively, which is comparable to other published studies from South Africa (33), but below the internationally achieved rates for living donors (34, 35).

The estimating equations for predicting kidney function that were assessed in this study performed poorly when compared to the gold standard radionuclear measured GFR. In this sample, the 4-v MDRD (with and without ethnicity adjustment) and the CKD-EPI (without ethnicity adjustment) equations significantly underestimated the average measured GFR (negative bias of -8 mL/min/1.73m^2, -16 mL/min/-1.73m^2 and -6.4 mL/min/1.73m^2 respectively). On the contrary, the bias associated with the average measured GFR using the CG and CKD-EPI (with ethnicity adjustment) equations was not significant (2.3 mL/min/1.73m^2 and 0.6 respectively).

The negative bias observed with each of the estimating equations was not proportionately distributed across all levels of measured GFR. In fact, the bias became more negative i.e. measured GFR was increasingly underestimated over the range of 50 -150mL/min/1.73m^2, irrespective of adjustments for ethnicity. This has important implications for the use of estimating equations to evaluate kidney function in healthy donors, where the expected GFR is high.

Currently in South Africa, the 4v-MDRD and the CKD-EPI equations are used by various clinical laboratories for reporting eGFR. Historically, the 4v-MDRD equation was developed in those with CKD and its poor performance in healthy donors could perhaps be explained. On the other hand, the CKD-EPI equation was developed to improve accuracy in those with better kidney function (>60 mL/min/1.73m^2) which makes its poor performance in this study more questionable.

It was interesting that the CG equation had the lowest bias for predicting the average measured GFR in this study, although this equation is no longer routinely used by laboratories. There are no comparable studies from sub Saharan Africa, but other published studies from Amsterdam, Korea and Pakistan found the CKD-EPI equation to have the least bias and best performance in predicting GFR; the average measured GFR in these studies ranged from 72.6mL/min/1.73m^2 to 120mL/min/1.73m^2 (36-38). However, in a study done at Cleveland clinic, U.S.A that evaluated the performance of creatinine based measures of GFR in a cohort of living kidney donors, the CG had less bias than the 4v-MDRD equation in predicting GFR (-0.5mL/min/1.73m2 and -11.0mL/min/1.73m^2 respectively), with a mean measured GFR of 106mL/min/1.73m^2 (39), this is similar to the findings of this study.

In order to explain the negative bias that was observed when predicting measured GFR, the impact of gender, age, weight and ethnicity was explored for each estimating equation. With respect to age, it was found that all GFR estimation equations showed a negative bias with increasing age. The implication for the older living donor is that if eGFR is used as the only measure of kidney function, they may not be accepted for donation even though their GFR may be appropriate. The question is whether it is fair to use a single cut off GFR for living kidney donation of all ages.

Some studies have proposed a correction factor for age which would be consistent

with the findings of our study, although we have not investigated the relationship between mGFR and increasing age. The British transplant society guidelines recommend a rigorous work up of older donors to ensure their suitability. The age-related decline in GFR has been accommodated by recommending GFR evaluation using a gold standard method and adjusting for age as per table 11.

Table 11. Acceptable GFR by donor age before donation

Donor age(yr)	Acceptable GFR before donation, corrected for BSA, (mL/min/1.73^2)
Up to 46	80
47-50	77
51-60	68
61-70	59
71-80	50

With respect to weight, the CG estimates were not affected by donor weight (as it adjusts for this in the formula). However, both 4v-MDRD and the CKD-EPI equations underestimated GFR with increasing donor weight. The implication for living kidney donation is that potential donors with a higher BMI are more likely to be rejected even though their mGFR may be within acceptable limits. This suggests the need for a weight correction factor in this population.

There was no effect of gender on the 4v-MDRD and CKD-EPI estimates in our study. However, the CG estimates showed a positive bias of 7 mL/min/1.73m^2 for female compared to male donors, thus the CG equation overestimated GFR in female compared to male donors. The reason for this is unclear, however, it may suggest that the gender factor used in the CG equation is not be optimal.

In Black compared to white South Africans, use of the ethnicity factor resulted in a significant overestimation of mGFR for the 4v-MDRD equation (by 24.2 mL/min/1.73m^2 compared to 6.8 mL/min/1.73m^2 without it). Similarly, the ethnicity factor significantly overestimated mGFR for the CKD-EPI equation (by 21.8 mL/min/1.73m^2, compared to 7.6 mL/min/1.73m^2, without the ethnicity factor). The implication for living kidney donation is that potential donors with sub-optimal kidney

function may be accepted for donation if screened using the 4v-MDRD and the CKD-EPI equations with ethnicity correction. In South Africa today, the laboratories use the CKD-EPI equation without adjustment for ethnicity, which is consistent with the findings of this study.

When comparing the performance of the eGFR equations in relation to mGFR and the clinical cut-off of 80 mL/min/1.73m^2, in this study, the 4v-MDRD equation (without adjustment for ethnicity) had the highest sensitivity for determining donors with GFR <80 mL/min/1.73m^2, while the CG equation had the highest specificity. Similar findings were reported in a U.K study which also demonstrated the 4v-MDRD to have a relatively higher sensitivity for identifying potential donors with GFR<80 mL/min/1.73m^2 compared to the CG and CKD-EPI equations (40). However, as in various other international studies, the sensitivity, specificity and positive predictive value of all four prediction equations for determining donors with GFR <80 mL/min/1.73m^2 was poor (38-41). In one study from India assessing the performance of GFR estimation equations in evaluating potential living kidney donors, none of the GFR estimation equations was found to have sufficient sensitivity or specificity to reliably predict donors with GFR=>80 mL/kg/1.73m^2 (42). Based on our findings therefore, we would not recommend the use of these equations as a sufficient method for screening potential kidney donors in South Africa.

8. Limitations of the study

Due to the retrospective nature of this study there were missing results that precluded a complete data set. While this remains a limitation, the levels of missing data have been included in all the results in this study to ensure completeness. The inability to evaluate the performance of the 24 hour urine creatinine clearance as a method of estimating GFR is another limitation. This was in part due missing data, but also due to the lack of uniformity in the work up testing of patients – as practice does vary between sites and not all potential donors perform this test. Inferences from this study may be limited as it was confined to one geographical region of the country. On this basis, the findings of this study may be used to inform future studies that would preferably be prospective and multi-centre in design.

9. Recommendations

Based on the findings of this study, the following recommendations may be helpful to inform policy on the process of living kidney donor evaluation in South Africa:

1. A gold standard measured GFR test is the preferred test for the evaluation of kidney function in all potential living kidney donors

2. Rather than a single cut-off value of 80 mL/min/1.73m^2, a correction factor for age should be considered when assessing GFR in potential living kidney donors, particularly in the older age group. The development of this correction factor could be informed by the data from this study.

3. In the preliminary screening of potential living kidney donors using estimating equations for GFR, the ethnicity correction factor should not be used in estimating GFR using the v-MDRD and the CKD-EPI equations

4. Further studies to determine need for a weight correction factor for the 4-vMDRD and the CKD-EPI equations as well as determining if the gender factor for the CG equation is optimal.

10. Conclusion

Most potential donors were young, female and of black ethnic origin, and almost half of the potential living donors who were evaluated were ineligible to donate. The most common reasons for ineligibility were obesity, hypertension, abnormalities of the urinary tract and low measured GFR. When comparing the performance of the eGFR equations to measured GFR, they all performed poorly, particularly at higher measured GFR. Based on the findings of this study, it is appropriate that measured GFR should be the gold standard for evaluating kidney function in potential living kidney donors in South Africa. This is in line with the British Transplant society guidelines which recommend that GFR in potential living kidney donors should be measured using a reference GFR procedure, for example, ^{51}Cr EDTA (43).

Appendix A

Ethical clearance certificate

R14/49 Dr Jacktone Okuthe et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140922

NAME: (Principal Investigator)	Dr Jacktone Okuthe et al
DEPARTMENT:	Internal Medicine WDGMC CHBAH
PROJECT TITLE:	Assessment of glomerular filtration rate in the evaluation of potential kidney donors at the (WDGMC) and (CMJAH)
DATE CONSIDERED:	03/10/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr June Fabian
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	22/05/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature — Date 7/07/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

PROTOCOL FOR CR51-EDTA GFR

1. Work out the different quantities of Cr 51-EDTA and saline – for e.g., 0.4mL Cr51- EDTA AND 5.6mL Saline – make enough for the amount of patients to be done, plus 1mL for the standard – mix in a vial.

2. Withdraw 1mL of the solution into a 1mL syringe, take the needle off and then weigh the syringe three times and record weights.

3. Dispense the contents of the standard in a 100mL flask.

4. Measure the empty syringe without the needle, three times, and record weights.

5. Add distilled water to the flask, till 100 mL, until the lower level of the meniscus.

6. Put parafilm over the top, and mix.

7. Take a 1 mL pipette and dispense 1mL in each of 3 labelled standard tubes – put date on.

8. Count standard tubes each for 10 mins. If the counts are around 5000 cpm, then the dose per patient is 5 mL. If the counts are higher, 6000 cpm, then give the patient 4 mL If the counts are lower, 4000 cpm, then give the patient 6mL.

9. Draw up the dose of the patient, weigh syringe without needle, three times and record weights.

10. Before you inject the patient, he/she must have a glass of water to drink, and they must continue to drink one glass of water every half hour during the entire test. The patient may empty their bladders when necessary. During the test they can eat, but NO PROTEIN or CAFFEINE.

11. Inject patient, record time, and in which arm the injection was given. Also record the weight and height of the patient – these measurements are necessary for analysis of the GFR.

12. Weigh empty syringe, without needle, three times and record weights.

13. Draw blood samples, from opposite arm to injection, at 3 hours post injection, into green-topped heparinised tubes. Draw three tubes. Record exact time when sample was drawn – important that the blood is drawn at exactly 3 hours post injection.

14. Spin each tube of blood in a centrifuge for 10mins.

15. Withdraw 1 mL of supernatant with a 1 mL pipette carefully, and put into a tube, labelled with the correct time of sample.

16. Try to withdraw 3 mLs of the supernatant into 3 different tubes.

17. Put the three standard tubes, the three 3hr samples into a rack, and count each tube for 10 mins, using protocol 2 clip.

18. Once counting has been completed, enter figures into excel spreadsheet to determine GFR – enter weights of dose and standard, enter weight and height of patient, enter name, age, sex, enter counts of standard and the two samples.

19. Print GFR result out and a report and fax or deliver to relevant doctor.

Appendix C

Potential donors who had m GFR data as well as data for each of the comparative eGFR measures

GFR measure	Number of cases
RN	286
MDRD-b	297
MDRD-e	274
CG	209
CKD-EPI-b	297
CKD-EPI-e	280
Main comparisons	**Number of cases**
RN vs. MDRD-b	233
RN vs. MDRD-e	213
RN vs. CG	154
RN vs. CKD-EPI-b	233
RN vs. CKD-EPI-e	213
Exploration of main comparisons	**Number of cases**
RN vs. MDRD-b	233
RN vs. MDRD-b with age	233
RN vs. MDRD-b with gender	233
RN vs. MDRD-b with ethnicity	213
RN vs. MDRD-b with weight	189
RN vs. MDRD-e	213
RN vs. MDRD-e with age	213
RN vs. MDRD-e with gender	213
RN vs. MDRD-e with ethnicity	213
RN vs. MDRD-e with weight	175
RN vs. CG	154
RN vs. CG with age	154
RN vs. CG with gender	154
RN vs. CG with ethnicity	144
RN vs. CG with weight	154
RN vs. CKD-EPI-b	233
RN vs. CKD-EPI-b with age	233
RN vs. CKD-EPI-b with gender	233
RN vs. CKD-EPI-b with ethnicity	213
RN vs. CKD-EPI-b with weight	189
RN vs. CKD-EPI-e	213
RN vs. CKD-EPI-e with age	213
RN vs. CKD-EPI-e with gender	213
RN vs. CKD-EPI-e with ethnicity	213
RN vs. CKD-EPI-e with weight	175

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