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Original Article

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Comparison between creatinine and cystatin C-based GFR equations in renal transplantation

Ahmed Zahran[1], Mabood Qureshi[2] and Ahmed Shoker[1]

[1]Department of Medicine, Division of Nephrology and [2]Department of Pathology, Royal University Hospital, University of Saskatchewan, Canada

Abstract

Background. Estimation of glomerular filtration rate (GFR) from serum creatinine (S_{cr}) or cystatin C (Cys C) exhibit variable performances.

Methods. We compared the performances of 14 S_{cr} and 9 Cys C estimated GFR equations using inulin clearance (Cl_{in}) as the reference test in 103 stable renal transplant populations. Bias, precision, receiving operation characteristics (ROC), accuracy within 30% ranges from the reference method and agreements of each test were compared.

Results. Mean Cl_{in} was 46.4 ± 20.9 ml/min/1.73 m^2. S_{cr} and Cys C levels correlated well with each other ($r = 0.83$, $P < 0.0001$) and with Cl_{in} ($r = -0.57$ and -0.53, $P < 0.001$, respectively). ROC analysis demonstrated no superiority of Cys C over S_{cr}. Gats equation achieved the highest accuracy of 70% in patients with GFR $\geq$60 ml/min/1.73 m^2. In patients with GFR $\geq$60 ml/min/1.73 m^2, the Nankivell equation demonstrated the highest accuracy of 73.91%. Cys C-based equations were not depicted to be thoroughly accurate. Bias, precision and agreement were otherwise similar in all GFR tests.

Conclusion. S_{cr}-based equations did not appear to be inferior to Cys C-based equations as a means to estimate GFR in renal transplant patients.

Keywords: cystatin C; GFR; renal transplantation; serum creatinine

Introduction

The present approach adopted to assess the renal function in humans is often limited to measurements of proxies for glomerular filtration rate (GFR) such as serum creatinine (S_{cr}), creatinine clearance (Cl_{cr}) and estimates of GFR derived from S_{cr}-based equations [1]. The limitations of S_{cr} and Cl_{cr} for estimation of GFR are well known. S_{cr} concentration is affected by several factors that are independent of changes in GFR such as age, race, muscle mass, gender, medication use and catabolic state [2]. Various S_{cr}-based equations have been developed in an attempt to improve the estimation of GFR from S_{cr} [3–16]. These equations, however, have not been shown to be accurate in renal transplant recipients, and their suitability in clinical trials has been called into question [17]. In addition, the methods used to measure S_{cr} interfere with the accuracy of the GFR estimation formulae [18]. To circumvent the problems attached to the measurement of GFR based on S_{cr}, several investigators studied the feasibility of cystatin C (Cys C) as a marker of GFR [19–21]. Recently, several prediction equations have been derived from both paediatric and adult patients to estimate GFR from the Cys C concentration [22–29]. However, only three studies tested the performance of GFR equations based on Cys C or S_{cr} levels in renal transplant patients [30–32]. In these studies, the reference method was an isotope GFR scan and they reached discrepant results. Two studies compared the performance of S_{cr} and Cys C concentration in renal transplant patients using inulin clearance (Cl_{in}) as a reference method. In these studies, no comparison of the current equations was performed [20,33]. Accordingly, the objective of this study was to compare the performance of GFR estimates from the Cys C and S_{cr} concentrations using old and recent equations in an independent sample of adult renal transplant recipients. Bias, precision, accuracy within 30% range from the reference GFR and agreement of the prediction equations were compared with Cl_{in}.

Materials and methods

Study population

The protocol for this study followed the ethical standards of this institution. Adult renal transplant recipients who were followed at the Saskatchewan Transplant Program and

Correspondence and offprint requests to: Ahmed Shoker, Division of Nephrology, St. Paul's Hospital, 1702 – 20th St. West, Saskatoon, SK S7M 0Z9, Canada. Email: shoker@sask.usask.ca

were at least 1-month post-transplantation and had stable renal function were eligible to participate. Consecutive patients, who met these criteria and had undergone all laboratory testing completed between August 2005 and July 2006, were included in this analysis. However, patients were excluded for the following reasons: unable or unwilling to provide informed consent, pregnant or breastfeeding women, acute rejection or a change in S_{cr} of >10% within the preceding 4 weeks or showed evidence of congestive heart failure or chronic liver disease associated with ascites.

Laboratory assessment

The inulin clearance (Inutest). GFR was measured by the Cl_{in} (Inutest), in which sinistrin, an inulin analogue, is used as a substitute for inulin because it is more water soluble and easy to handle. The procedure started at 8.30 a.m. after an overnight fasting. Two I.V. lines were established, one for injection and the other for sampling. Hydration was achieved with loading with oral water (10 ml/kg) to produce good urine volume of at least 2 ml/min. A blank urine sample was voided and a blood sample was drawn, followed by injection of an intravenous loading dose of Inutest® 25% (Fresenius, Linz, Austria) over 1 minute. The dose was calculated from the necessary plasma concentration (250 mg/l) and the sinistrin volume of distribution as recommended by the company as follows:

$$\text{Bolus dose (mg)} = \text{Required plasma concentration} \times \text{Estimated sinistrin volume of distribution.}$$

The sinistrin volume of distribution approximately corresponds to the extracellular volume and amounts to ~18% of the body weight.

The bolus dose was followed immediately by a maintenance dose to achieve sinistrin plasma concentration of approximately 250 mg/l as follows:

$$\text{Maintenance infusion dose (mg/min)} = \text{Required plasma concentration (mg/l)}/1000 \times \text{Estimated clearance by Cockcroft} - \text{Gault formula.}$$

This dose was infused over 160 min in 250 ml normal saline by IVAC pump.

After an equilibration period of 90 min, patients were asked to empty their bladder spontaneously and then two consecutive clearance periods of 30 min each were analysed. During each period, urine volume was collected, measured and sampled. Blood samples were drawn at the beginning and end of each period (the sample at the end of the first period was considered the beginning sample for the second period). Cl_{in} was measured as the mean of the two clearance periods with the formula UV/P, where U and P are sinistrin concentration in urine and plasma and V is urine flow rate ml/min. Cl_{in} was expressed in 1.73 m^2. Body surface area was calculated by Du Bois formula [34].

Inulin concentrations were determined by Heyrovsky method [35]. In the original method, each sample was read over approximately 15 s in a cuvette by a spectrophotometer. We found that the absorbance of the reaction increased in a time dependant fashion (data not shown) Therefore, 0.25 ml triplicate samples were measured in 96 round U bottom microplate wells (Evergreen Scientific). Samples were measured on a UV at 520 nm using microplate reader (ELx 808™ Absorbance microplate reader, BIO-TEK). With this modification, sinistrin concentration of 0.02–0.5 mg/ml and optic density remained linear with an r^2 of >0.98. It should be noted that all measured samples had concentrations well within this range. Elevated blood sugar is known to affect the *in vitro* concentration of sinistrin. In our preliminary analysis, we confirmed that blood sugar up to 12 mmol/l does not affect our assay, and therefore, we included diabetic transplant patients who demonstrated a blood sugar level of <12 mmol/l prior to the procedure. Our modified method has an intra-assay CV of <6% and an inter-assay CV of consistently <8%. The 103 Cl_{in} retained for this analysis were all performed on patients who maintained a urine flow rate of at least 2 ml/min and established a stable sinistrin concentration that did not vary by >10% in the three plasma samples.

Measurement of cystatin C concentrations

Cys C was measured by Enzyme Linked Immunosorbent Assay (ELISA), (Biovender Laboratory Medicine, Inc.) using the microplate reader (ELx 808™ Absorbance microplate reader, BIO-TEK) at wave length 450 nm. The intra-assay CV was 9.6% at 589 ng/ml (0.589 mg/l) and 5% at 2862 ng/ml (2.862 mg/l). The inter-assay CV was 6.2% at 600 ng/ml (0.6 mg/l) and 4.8% at 2905 ng/ml (2.905 mg/l). This ELISA method has an r of 0.97 (r^2 0.94) when compared with the DADE B latex assisted turbidimetry method and r of 0.96 (r^2 0.92) with DAKO ELISA method as studied by the manufacture (personal communication).

Measurement of serum creatinine concentrations

Serum creatinine was measured with an enzymatic assay (SYNCHRON LX 20 Systems, Bachman, Coulter, Inc., Fullerton, CA, USA) with a normal range of 60–110 μmol/l. The intra-assay and inter-assay CV were <3%. This method has a small bias of $\leq +6$ umol/l as compared with the isotope dilution mass spectrometry (IDMS), however, this difference is not considered significant in estimation of GFR [36].

GFR estimates

Fourteen S_{cr}-based equations [3–16] and nine Cys C-based equations [22–29] (Table l) were studied. We included the modified MDRD equation which is suggested for use to estimate GFR [13] when S_{cr} is measured by IDMS method. Continuous data were presented as mean ± SD and discrete variables as frequency (%). A P value of <0.05 was considered as statistically significant.

Statistical evaluation of the predictive formulas

We used the statistical package of social signs (SPSS, version 14) and Excel to perform the analysis. The evaluation of the prediction equations was performed by calculating the bias, precision, agreement and accuracy as recommended in the National Kidney Foundation guidelines on chronic kidney disease [37]. In addition, beta errors between all equations and Cl_{in} were calculated. Bias was defined as the mean difference between the measured and estimated GFR. Precision was defined as SD of the difference between the measured and estimated GFR. Accuracy was defined as the

Table 1. Serum creatinine and cystatin C-based prediction equations

Reference	Formulae
S_{cr}-based equations	
Cockcroft–Gault[a] [3]	$\frac{(140-\text{Age})}{72\times S_{cr}}(\text{Wt}) \times (0.85 \text{ for female})$
Bjornsson[a] [4]	For males: $\frac{[27-(0.173\times\text{Age})]\times\text{Wt}\times 0.7}{S_{cr}}$
	For females: $\frac{[25-(0.175\times\text{Age})]\times\text{Wt}\times 0.7}{S_{cr}}$
Davis[a,c] [5]	$\frac{140-\text{Age}}{S_{cr}} \times (0.85 \text{ for female})$
Edwards[a,c] [6]	For males: $\frac{94.3}{S_{cr}} - 1.8$
	For females: $-\frac{69.9}{S_{cr}} + 2.2$
Gates[a] [7]	For males: $89.4 \times S_{cr}^{-1.2} + (55 - \text{Age}) \times 0.447 \times S_{cr}^{-1.1}$
	For females: $60 \times S_{cr}^{-1.1} + (56 - \text{Age}) \times 0.3 \times S_{cr}^{-1.1}$
Hull[a] [8]	$\frac{(145-\text{Age}-3)}{S_{cr}} \times (0.85 \text{ for female})$
Jelliffe[a] [9]	$\frac{(98-0.8(\text{Age}-20))}{S_{cr}} \times (0.90 \text{ for female})$
Jelliffe[a] [10]	For males: $\frac{100}{S_{cr}} - 12$
	For females: $\frac{80}{S_{cr}} - 7$
Mawer[b] [11]	For males: $\frac{\text{Wt}\times(29.3-0.203\times\text{Age})\times[1-(0.03\times S_{cr})]}{(1.44\times S_{cr})\times(70/\text{Wt})}$
	For females: $\frac{\text{Wt}\times(25.3-0.175\times\text{Age})\times[1-(0.03\times S_{cr})]}{(1.44\times S_{cr})\times(70/\text{Wt})}$
MDRD2[a] [12]	$186 \times (\text{Scr})^{-1.145} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African} - \text{American})$
MDR 2[a,d] (IDMS) [13]	$175 \times (\text{Scr})^{-1.145} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African} - \text{American})$
Nankivell[b] [14]	$(6.7/\text{Scr}) + (\text{Wt}/4) - (\text{Urea}/2) - (100/\text{height}^2) + [35 \text{ for male or } 25 \text{ for female}]$
Salazar[a] [15]	For males: $\frac{(137-\text{Age})\times[(0.285\times\text{Wt})]+(12.1\times\text{Height}^2)}{(51\times\text{Scr})}$
	For females: $\frac{(140-\text{Age})\times[(0.285\times\text{Wt})]+(12.1\times\text{Height}^2)}{(60\times\text{Scr})}$
Walser[b,e] [16]	For male: $\frac{7.57}{S_{cr}} - 0.103 \times \text{Age} + 0.096 \times \text{Wt} - 6.66$
Cys C-based equations	For female: $\frac{6.06}{S_{cr}} - 0.08 \times \text{Age} + 0.08 \times \text{Wt} - 4.81$
Le Bricon[c] [22]	$78 \times 1/\text{Cys C} + 4$
Hoek[c] [23]	$-4.32 + 80.35/\text{Cys C}$
Filler[f] [24]	$1.962 + 1.132 \times \text{Log}(1/\text{Cys C})$
Larsson [25]	$77.24 \times \text{Cys C}^{-1.2623}$ (Dade Behring Cys C calibration) $99.43 \times \text{Cys C}^{-1.5837}$ (DakoCytomation Cys C calibration)
Grubb [26]	$99.19 \times \text{Cys C}^{-1.713} \times (0.823 \text{ for women}).$
Grubb[c] [27]	$86.49 \times \text{Cys C}^{-1.686} \times (0.948 \text{ if female}).$
Macias [28]	84.6/Cys C – 3.2
Rule [29]	$76.6 \times \text{Cys C}^{-1.16}$

Age in years (observed at time of study); Wt, weight in kilograms; height in metres. Urea, mmol/l; MDRD, modification of diet in renal disease; $S_{cr.}$ serum creatinine; Cys C, cystatin C (mg/l).
[a]Serum creatinine (mg/dl).
[b]Serum creatinine (mmol/l).
[c]GFR corrected to body surface area.
[d]MDRD equation with creatinine calibrated to isotope dilution mass spectrometry (IDMS).
[e]GFR corrected for $3\,m^2$.
[f]Formula for log GFR.

percentage of GFR estimates lying within 30% of measured GFR. In addition, the limits of agreement between the estimated and measured GFR were presented in tabulated and graphic forms using Bland and Altman analysis (Figures 2 and 3) [38]. Moreover, we performed receiving operation characteristics (ROC) analysis to substantiate our results. ROC analysis was performed to quantitate the accuracy of S_{cr} and Cys C to detect reduced GFR using a cut-off value of 30 and 60 ml/min/1.73 m^2. We used MedCalc software to find if there is a significant difference between area under the curve (AUC) for S_{cr} and Cys C.

Results

Of the 120 patients approached, a total of 110 stable renal transplantations agreed to participate in

Table 2. Patient characteristics

Variable	*n*	Mean ± SD	Range
Inulin clearance (ml/min/1.73 m^2)	103	46.4 ± 20.7	12.3–121.6
Gender: male	63 (61%)		
Gender: female	40 (39%)	47.51 ± 13.62	18.00–76.00
Age (years)	103	81.62 ± 20.94	44.50–140.00
Weight (kg)	103	1.71 ± 0.10	1.44–1.96
Height (metre)	103	1.93 ± 0.27	1.39–2.56
Body surface area	103	60.41 ± 47.47	1.00–204.00
Tx Duration (months)	103	147.84 ± 60.03	74.00–406.00
Serum creatinine (μmol/l)	103	10.36 ± 4.98	3.60–33.80
Urea (mmol/l)	103	1.96 ± 0.87	0.74–6.08
Serum cystatin C (mg/l)	101		

Table 3. Patient characteristics with GFR >60 ml/min/1.73 m^2

Variable	*n*	Mean ± SD	Range
Inulin clearance (ml/min/1.73 m^2)	23	77.13 ± 13.69	60.30–121.59
Gender: male	14 (61%)		
Gender: female	9 (39%)		
Age (years)	23	47.74 ± 13.86	22.00–71.00
Weight (kg)	23	85.75 ± 23.31	44.50–128.20
Height (metre)	23	1.68 ± 0.10	1.56–1.91
Body surface area	23	1.94 ± 0.28	1.41–2.40
Tx Duration (months)	23	54.96 ± 28.53	1.00–102.00
Serum creatinine (μmol/l)	23	109.52 ± 25.36	74.00–163.00
Urea (mmol/l)	23	7.82 ± 2.50	3.60–12.70
Serum cystatin C (mg/l)	22	1.51 ± 0.48	0.82–2.43

this study. Seven patients were excluded. Two patients developed an allergy requiring discontinuation of the test, two patients produced a urine flow rate of <2 ml/min and three patients did not achieve a steady state as recognized from a significant difference in sinistrin plasma concentrations of >10% and 72% of patients had received a kidney from a cadaveric donor. Causes of end-stage renal disease were non-diabetic glomerular diseases in 61%, diabetic glomerular disease in 24% and congenital and unknown in 15%. Mean urine flow rate during Cl_{in} procedure was 6.6 ml/min. (medium 6.2; range 2–15). The baseline characteristics of the cohort and subgroups with GFR above and below 60 ml/min/1.73 m^2 are presented in Tables 2–4. The majority of the patients included in the study were males. The patients had a wide range of renal function that encompassed all five stages of kidney disease, outcomes, quality initiative and chronic kidney disease classification system [39]. All patients were on prednisolone, calcineurin inhibitors in addition to a mycophenolate derivative (CellCept or Myfortic) or rapamune. All patients were on Septra one tablet every other day. Tables 2–5 show the statistical descriptive results. The average Cl_{in} was 46.4 ± 20.7 ml/min/1.73 m^2, with a range of 12.3–121.6 ml/min/1.73 m^2 and coefficient of variation

Table 4. Patient characteristics with GFR <60 ml/min/1.73 m^2

Variable	*n*	Mean ± SD	Range
Inulin clearance (ml/min/1.73 m^2)	80	37.57 ± 12.09	12.30–59.34
Gender: male	49(61%)		
Gender: female	31(39%)		
Age (years)	80	47.45 ± 13.69	18–76
Weight (kg)	80	88.44 ± 20.21	45–140
Height (metre)	80	1.72 ± 0.10	1.44–1.96
Body surface area	80	1.93 ± 0.26	1.39–2.56
Tx Duration (months)	80	61.98 ± 51.69	1.00–204
Serum creatinine (μmol/l)	80	158.86 ± 62.64	75–406
Urea (mmol/l)	80	11.09 ± 5.58	4.5–33.8
Serum cystatin C (mg/l)	79	2.08 ± 0.92	0.74–6.08

n, number of patients; Tx, transplantation.

Table 5. Mean, median and range of measured and estimated GFR

Equations	*n*	Mean ± SD	Median	Range
Inulin clearance (ml/min/1.73m^2)	103	46.4 ± 20.7	42.86	12.3–121.6
S_{cr} based equations				
Cockcroft–Gault	103	58.4 ± 20.7	57.59	11.6–135.9
Bjornsson	103	60.8 ± 21.3	60.70	12.6–138.1
Davis	103	58.3 ± 19.2	57.71	14.3–107.7
Edwards	103	56.2 ± 17.5	54.97	18.8–103.8
Gates	103	46.2 ± 17.9	44.1	11.0–119.0
Hull	103	54.7 ± 20.5	53.17	14.4–130.0
Jelliffe 1	103	45.1 ± 17.6	43.66	11.6–110.6
Jelliffe 2	103	47.2 ± 19.1	46.29	7.4–108.0
Mawer	103	71.6 ± 38.8	62.48	9.2–250.9
MDRD 2	103	44.6 ± 17.1	43.80	10.9–108.9
MDRD 2 (IDMS)	103	47.8 ± 20.1	45.92	10.2–124.22
Nankivell	103	56.5 ± 15.7	56.96	19.0–101.5
Salazar	103	57.5 ± 19.0	58.10	14.8–107.1
Walser	103	51.6 ± 18.2	51.80	9.5–98.8
Cys C-based equations				
Le Bricon	101	50.6 ± 17.8	49.87	16.8–108.7
Hoek	101	43.7 ± 18.3	42.93	8.9–103.6
Filler	101	47.7 ± 23.2	42.49	8.7–135.5
Larsson (Dade Behring)	101	38.1 ± 20.5	33.24	6.0–117.8
Larsson (DakoCytomation)	101	43.3 ± 29.0	36.50	4.3–167.0
Grubb 1	101	38.1 ± 27.3	31.79	3.4–159.5
Grubb 2	101	38.5 ± 25.1	35.33	4.1–134.7
Macias	101	43.7 ± 20.1	39.88	8.1–116.1
Rule	101	39.4 ± 19.6	34.96	7.2–113.5

n, number of patients; S_{cr}, serum creatinine; Cys, cystatin C; MDR, modification of diet in renal disease; IDMS, isotope dilution mass spectrometry.

of 44.6%. The mean, median and range of estimated GFR with the different prediction equations are shown in Table 5.

Performance of S_{cr} and Cys C

Pearson's correlation coefficients (*r*) between normalized Cl_{in} and S_{cr} or Cys C showed a significant,

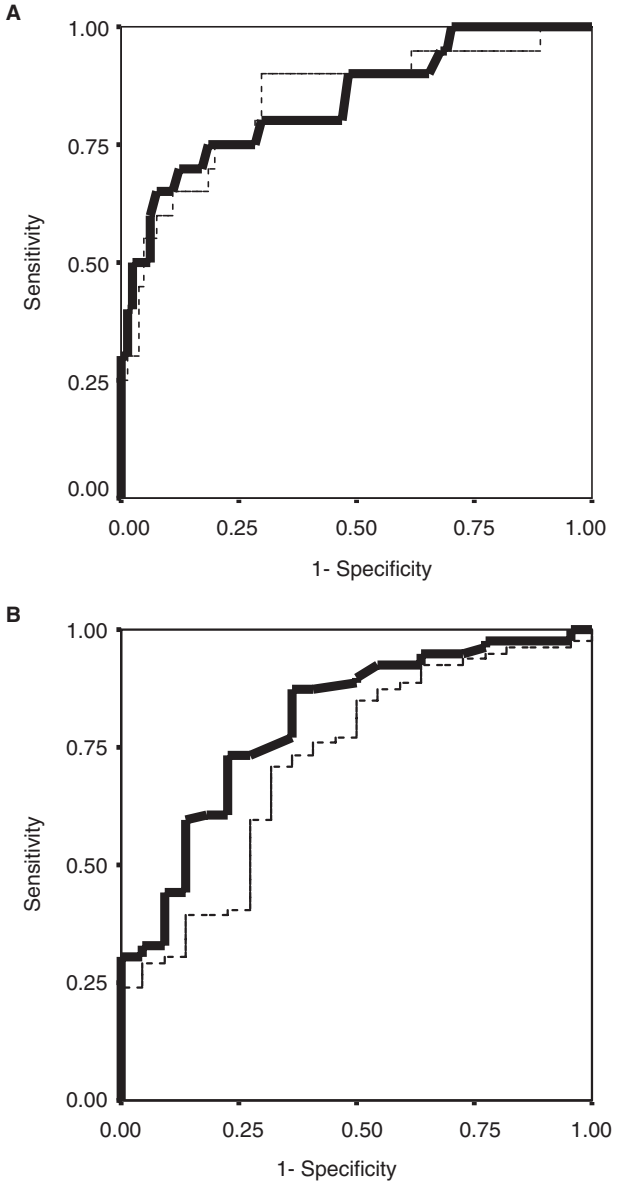

Fig. 1. Non-parametric ROC plots for the diagnostic accuracy of serum creatinine concentration (solid line) and cystatin C (dotted line) in reduced GFR (<30 ml/min/1.73 m^2). (**A**) The area under the curve for serum creatinine = 0.842, SE = 0.054, $P = 0.000$ and for cystatin C = 0.841, SE = 0.054, $P = 0.001$. (**B**) The area under the curve for serum creatinine = 0.803, SE = 0.052, $P = 0.000$ and for cystatin C = 0.718, SE = 0.061, $P = 0.001$.

negative correlation between normalized Cl_{in} with S_{cr} and Cys C with r value −0.57 ($r^2 = 0.32$) and −0.53 ($r^2 = 0.28$), respectively. Of more importance was the observation that S_{cr} and Cys C levels correlated with an r of 0.83 ($r^2 = 0.69$) and $P < 0.0001$, which suggest a similar performance of both molecules to predict GFR in this population. Figure 1A and B show that the area under the ROC curves (95% confidence interval) at cut-off value of 30 ml/min/1.73 m^2 for S_{cr} and Cys C were 0.842 (0.736–0.947) and 0.841 (0.735–0.948), respectively. At 60 ml/min/1.73 m^2 these values were 0.803 (0.736–0.905) and 0.718 (0.598–0.839), respectively. Again, there was no significant difference between Cys C and S_{cr} at cut-off values 30 ml/min/1.73 m^2 and 60 ml/min/1.73 m^2 to detect changes in GFR ($P = 0.995$ and 0.080, respectively).

Performance of the derived mathematical equations in total populations

The Gats equation was the most accurate. It had the least bias (−0.24) and the highest percentage of values that fell within 30% of the Cl_{in} (66.02%). The MDRD 2 equation had the second best accuracy in the general population with a reasonable bias of −1.68 ml/min and accuracy of 65.05%. Among the Cys C-based equations, the Hoek had the best accuracy of 55.45% and the Filler equation had the least bias of 1.44 ml/min. Each GFR test correlated significantly ($P = 0.0001$) with Cl_{in}. Analysis of r^2 pointed out that only 41% of the interindividual variability for GFR prediction by Edwards' formula was explained by true difference in Cl_{in}, while it was 26% for the Hoek equation. For all equations, <25% of variance was explained by the estimation formulae. Limits of agreement between the predicted and measured tests showed lack of reliable agreement as presented in Table 6.

Performance of the derived mathematical equations in patients with GFR above and below 60 ml/min/1.73 m^2

Table 7 shows the performance of the GFR equations in this subgroup. In patients with GFR above 60 ml/min/1.73 m^2, the Nankivell and Hull equations were the most accurate GFR estimates, while C–G equations demonstrated the least bias among all GFR estimates. Analysis of accuracy showed that the performance of Cys C-based equations were approximately 20% lower than that of the creatinine based equations in patients with GFR below 60 ml/min/1.73 m^2. The Gats equation followed by MDRD 2 showed the highest accuracy. The MDRD 2 showed the least bias followed by Jellife 1 and Gats equations. Among the Cys C-based equations, Macisac followed by Hoek had the highest accuracy. Bias was the least in the Larsson equation (DakoCytomation).

Discussion

In a recent international survey, opinion on GFR estimation was evaluated. The majority used either the Cockcroft–Gault formula or the MDRD 2 equation [39]. Therefore, these formulae as well as other tests of GFR equations were included to insure complete comparison between the old and recently available equations. The comparison of these equations was extended to patients with GFR above and below 60 ml/min/1.73 m^2 in accordance with chronic kidney disease classifications cut-off limit.

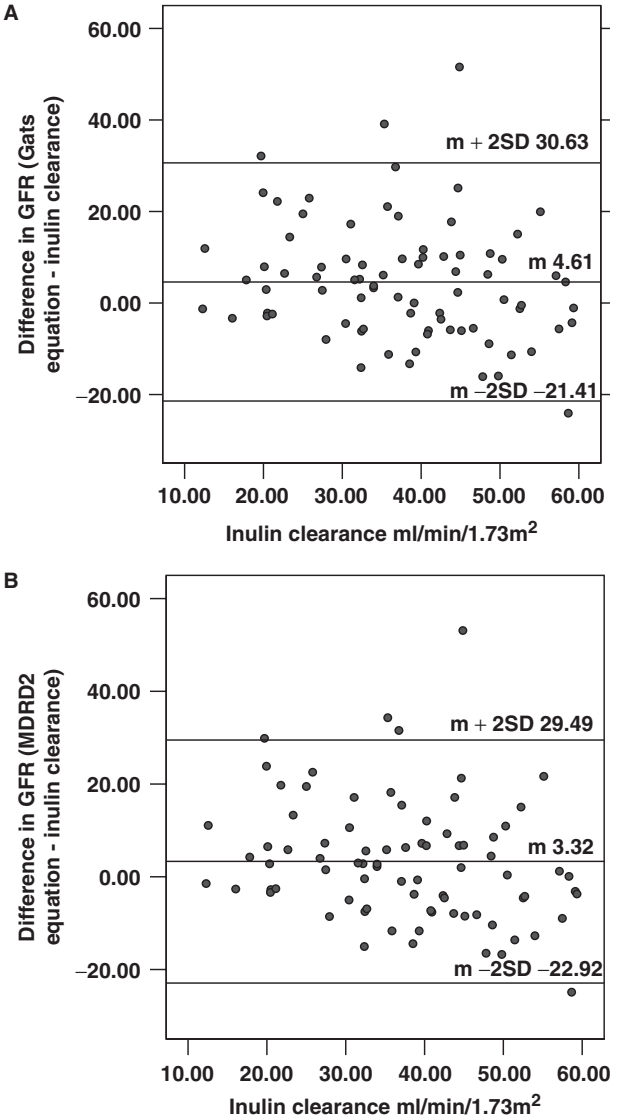

Fig. 2. Bland and Altman analysis of GFR estimates. In this graphical method the difference between predicted and measured GFR is plotted against inulin clearance. The mean difference (*m*) is indicated by centre line, limits of agreement are indicated by the upper ($m+2$SD) and lower ($m-2$SD) lines. Gats and Cl_{in} are plotted in (**A**), MDRD 2 and Cl_{in} are plotted in (**B**).

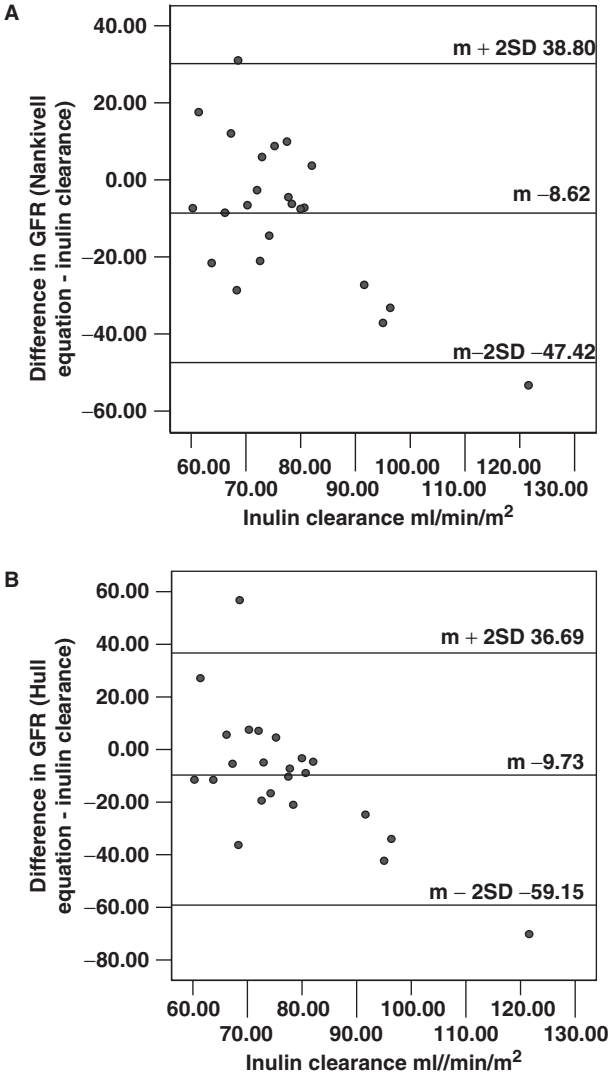

Fig. 3. Bland and Altman analysis of GFR estimates. In this graphical method the difference between predicted and measured GFR is plotted against inulin clearance. The mean difference (m) is indicated by centre line, limits of agreement are indicated by the upper (m + 2SD) and lower (m − 2SD) lines. Nankivell and Cl_{in} are plotted in (**A**) and Hull and Cl_{in} are plotted in (**B**).

The correlation coefficients for both S_{cr} and Cys C with Cl_{in} nearly showed similar *r* values. In addition, the *r* value between Cys C and S_{cr} was highly significant, similar to that reported by Poge *et al.* [40]. ROC analysis in this study showed that S_{cr} is not inferior to Cys C as a surrogate marker for Cl_{in}. These results are at variance to those reported by Plebani *et al.* [33] who concluded the superiority of Cys C in a small group of 12 transplant patients. Our results are, however, in agreement with Daniel *et al.* [20] who performed 103 Cl_{in} in a larger cohort of 60 transplant patients. Our results are also in agreement with those of Poge *et al.* [40] and Christensson *et al.* [19] who found no difference in the ROC for Cys C (0.094) and S_{cr} (0.09). In total, these results suggest that there is no real difference in the diagnostic accuracy.

Consistent with other investigators, we noticed that patients with higher GFRs had a lower *r* value [41,42] albeit with a higher *r* value than ours. Our results are in agreement with the general notion that a decrease in GFR can occur below normal values by two thirds or more before an appreciable increase in serum creatinine occurs [43,44].

The accuracy of the S_{cr} and Cys C-based equations varied from one study to another [17,30–32,45] even in the recent literature in which isotope GFR was used. In this analysis, the Gates equation showed the highest accuracy in the general population and in patients with a GFR of <60 ml/min/1.73 m^2. The MDRD 2 and Jelliffe 1 were second preferred. The accuracy of the

Table 6. Limits of agreement of glomerular filtration rate prediction equations in total population

Equations	*n*	Lower limit	Upper limit
S_{cr}-based equations			
Gates	103	−36.38	35.90
MDRD 2	103	−38.10	34.38
Jelliffe 1	103	−40.63	37.93
Jelliffe 2	103	−28.98	39.38
MDRD 2 (IDMS)	103	−40.86	43.58
Walser	103	−37.35	39.01
Hull	103	−33.31	49.81
Salazar	103	−25.30	48.94
Edward	103	−23.34	42.86
Nankivell	103	−24.57	44.67
Davis	103	−24.32	48.08
Cockcroft–Gault	103	−24.71	48.65
Bjornsson	103	−22.89	51.63
Mawer	103	−42.97	93.35
Cys C-based equations			
Hoek	101	−41.58	36.42
Macias	101	−45.85	40.59
Filler	101	−45.28	48.16
Rule	101	−49.65	35.99
Le Bricon	101	−34.26	42.94
Larsson (Dade Behring)	101	−51.90	35.54
Larsson (DakoCytomation)	101	−58.04	52.08
Grubb 1	101	−59.95	43.65
Grubb 2	101	−55.61	39.99

n, number of patients; S_{cr}, serum creatinine; Cys C, cystatin C; MDRD, modification of diet in renal disease; IDMS, isotope dilution mass spectrometry.

Nankivell equation was particularly superior in patients with a GFR above 60 ml/min/1.73 m^2 a fact that was not identified previously in a similar work of Mariat *et al*. [17] who did not perform subgroup analysis. The Hull equation had a similar superior performance but with a slightly inferior bias to Nankivell.

Consistent with previous work [17,30,45] assessment of agreement indicated that none of the equations demonstrated an acceptable agreement with Cl_{in}, since they concealed a discrepancy of 23–93 ml/min/1.73m^2. It should be noted, however, that the agreement ranges concluded in this analysis are close to those concluded by Mariat *et al*. [17].

The findings of this study are clinically relevant given the previous research that showed conflicting results on the relative performances of S_{cr} and Cys C in kidney transplant recipients. Similar to S_{cr}, there are several reports which suggest that factors other than GFR may indeed affect Cys C concentration [46–51]. Therefore, Cys C may indeed suffer from limitations unseen previously, which questions its appeal as a replacement marker for S_{cr} to estimate GFR.

Comparison of Cys C and S_{cr} in transplant populations gave conflicting results [19–22,33,52–59]. Three studies were conducted with the intent to compare the performance of S_{cr} and Cys C-based equations in transplant patients. In one of the studies performed on 29 patients using ^{125}I-Iothalamate as the reference method, the authors concluded equal performances between the simplified MDRD and Larsson equation with an overall accuracy of 66% and 69%, respectively, within a 30% range from the reference method. The respective biases were 1.7 and 4.7 [30]. In the second transplant study, 117 transplant patients were included using ^{99m}Tc-DTPA as the reference method. In this study White *et al*. [31] showed that the accuracy ranges were exceptionally high for both S_{cr}-and Cys C-based equations. They concluded that Filler and Le Bricon equations performed the best, with 30% accuracy of 87% and 89% and bias of −1.7 and −3.8, respectively. In addition, some of the S_{cr}-based equations (Cockcroft–Gault, MDRD and Nankivell) performed better than some of the Cys C-based equations (Larsson showed the least accuracy of all equations). While in the third study, Poge *et al*. [32] showed that the Filler equation performed the least when compared with the Larsson, Hoek and MDRD equations. Furthermore, while the Larsson equation showed the highest performance, it was marginally better with only 10% superiority over the MDRD equation in regard to a 30% accuracy. Inconsistencies between these reports can be attributed to different isotope scan techniques among different centres. Current means to assess GFR in humans is limited to measurement of proxies for GFR such as S_{cr} and mathematically estimated GFR. Cys C, which has recently been proposed as another accuracy measure for GFR, was favoured in a number of studies over S_{cr} (reviewed in [60]). Comparison of Cys C and S_{cr}-based equations gave conflicting results. Although most of the studies favoured Cys C-based equations over S_{cr}-based equations, there was a lack of uniform superiority of these Cys C-based equations. Of more importance, we could not identify a consistent superiority of one equation over others in all the studies. Also, there was no consistency in the results in regard to correlation, bias, precision, accuracy and limits of agreement.

There are several inherent limitations to the use of Cys C-based GFR equations. Cys C was shown to be influenced by high-dose steroids. As such, Cys C level may be affected by differences in the steroid doses among patients [47,48]. It should be noted, however, that all our patients were on maintenance, small dose of prednisone of <10 mg daily. Risch *et al*. [47] showed, however, that renal transplant recipients on low-dose prednisone (5–10 mg/day) had a higher Cys C concentration compared with those on steroid-free immunosuppression. In contrast, Bökenkamp *et al*. [61] found no correlation between Cys C and the steroid dose. In addition, we did not simultaneously measure thyroid function to rule out hypothyroidism or hyperthyroidism, both of which can influence Cys C concentration [50]. However, we evaluated thyroid function as part of pretransplant work. Further, we are not aware of any recent study which compares isotope GFR to Cl_{in} using the current statistical methods including accuracy, precision and bias. These factors may explain, at least in part, some of the inconsistencies between these results and add more relevance to

Table 7. Precision, bias and accuracy of prediction equations compared with inulin clearance of GFR above and below 60 ml/min/1.73 m^2

GFR >60 ml/min/1.73 m^2 Equations	Precision	Bias	30% Accuracy	GFR <60 ml/min/1.73 m^2 Equations	Precision	Bias	30% Accuracy
S_{cr}-based equations				S_{cr}-based equations			
Nankivell	19.40	−8.62	73.91	Gates	13.01	4.61	70.00
Hull	24.71	−9.73	73.91	MDRD 2	13.11	3.35	68.75
Davis	23.38	−3.91	69.56	Jelliffe 1	14.25	4.50	66.25
Bjornsson	28.26	2.78	65.22	Jelliffe 2	13.16	5.59	60.00
Salazar	23.28	−5.01	65.22	MDRD 2 (IDMS)	17.13	6.57	60.00
Cockcroft–Gault	27.29	−0.04	60.87	Walser	13.35	9.34	57.50
Jelliffe 2	22.27	−15.75	60.87	Hull	16.32	13.42	47.50
MDRD 2 (IDMS)	23.90	−19.86	60.87	Edwards	12.03	14.12	46.25
Edwards	21.30	−5.35	56.52	Salazar	13.09	15.71	45.00
Walser	20.90	−9.16	52.17	Cockcroft–Gault	13.02	15.53	43.75
Gates	22.68	−17.11	52.17	Nankivell	12.30	15.14	41.25
MDRD 2	21.64	−19.68	52.17	Davis	13.31	16.42	41.22
Jelliffe 1	22.40	−21.72	47.83	Bjornsson	14.26	17.70	35.00
Mawer	54.46	24.85	47.83	Mawer	25.91	25.29	32.50
Cys C-based equations				Cys C-based equations			
Le Bricon	20.28	−16.93	50.00	Macisac	15.95	3.29	59.49
Hoek	20.66	−23.54	45.45	Hoek	14.63	3.26	58.23
Filler	28.07	−25.50	45.45	Filler	18.62	6.90	56.96
Rule	29.05	−18.18	40.91	Rule	15.62	−0.86	54.34
Grubb 2	35.59	−19.69	36.36	Larsson (Dade Behring)	16.24	−2.34	54.43
Larsson (DakoCytomation)	25.94	−23.87	36.36	Le Bricon	14.26	10.26	53.16
Macisac	34.92	−24.76	36.36	Larsson (DakoCytomation)	23.02	1.56	48.10
Grubb 1	25.69	−28.30	27.27	Grubb 1	16.86	−3.52	48.10
Larsson (Dade Behring)	26.59	−29.17	27.27	Grubb 2	20.19	−2.89	43.04

S_{cr}, serum creatinine; Cys C, cystatin C; MDRD, modification of diet in renal disease; IDMS, isotope dilution mass spectrometry. Equations arranged from highest to lowest accuracy.

the importance of Cl_{in} as a reference method to settle the controversy between S_{cr}-and Cys C-based equations. Of note, inulin was used as the comparison reference test in only two transplant studies [20,33]. In the larger study ROC analysis showed no superiority of Cys C over S_{cr} and the authors concluded that Cys C is not a more sensitive marker than S_{cr} or Cockcroft–Gault equation for detecting renal failure in transplant patients [20]. None of these two studies compared the performance of the mathematical GFR equations.

There are limitations to this study. The methods used to measure S_{cr} differ among centres. In addition, evaluation of the MDRD 2 equation requires calibration of the S_{cr} to the laboratory used in the MDRD 2 study, which was not done in this study. A small systematic error in S_{cr} measurement could greatly affect the results of a GFR [62]. The S_{cr} values were measured at the same laboratory using the same method to avoid differences in calibration. Our method has a small reported bias, however. Hallan *et al*. [63] pointed out that the bias due to a missing calibration decreases as S_{cr} increases. This is crucial since our cohort comprises a considerable percentage of patients with elevated S_{cr}. In addition, we added the suggested MDRD 2 (IDMS) in our comparative analysis. Another limitation is the small number of 23 patients included in this analysis with GFR above 60 ml/min/m^2. It is known, however, that most transplant patients have a modest GFR and as such our patient population represents the average transplant patients.

In conclusion, based on the data presented in a large renal transplant population, these S_{cr} and Cys C-based GFR tests exhibited a considerable lack of agreement with the reference GFR. Although, some equations demonstrated a better accuracy than others, we concluded that none of these formulae seemed good enough to safely substitute for Cl_{in} for point estimates of GFR, but are acceptable for discrimination of patients with chronic transplant kidney disease. Within these limitations, the data shows that Nankivell and Hull are the most performant formulae in patients with GFR above 60 ml/min/1.73 m^2, and the Gats and MDRD 2 demonstrated the best accuracy in patients with GFR below 60 ml/min/1.73 m^2. The data also confirmed lack of superiority of the current Cys C based equations over S_{cr}-based equations to estimate GFR in the renal transplant patient. Because of the high correlation between S_{cr} and Cys C, we do not see a merit for Cys C over S_{cr}-based equations as an ideal method to determine renal transplant function.

Conflicts of interest statement. None declared.

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