Nephrol Dial Transplant (2007) 22: 3013–3020
doi:10.1093/ndt/gfm318
Advance Access publication 7 June 2007

Original Article

NDT
Nephrology Dialysis Transplantation

Chronic kidney disease stage in renal transplantation—classification using cystatin C and creatinine-based equations

Christine White[1], Ayub Akbari[2,3], Naser Hussain[2], Laurent Dinh[4], Guido Filler[5], Nathalie Lepage[6] and Greg A. Knoll[2,3,7]

[1]Division of Nephrology, Queen's University, Kingston, [2]Division of Nephrology, University of Ottawa, Ottawa, Ontario, [3]Kidney Research Centre, The Ottawa Health Research Institute, Ottawa,[4]Division of Nuclear Medicine, Department of Medicine, [5]Division of Nephrology, Department of Pediatrics, Children's Hospital of Eastern Ontario, [6]Department of Laboratory Medicine, Children's Hospital of Eastern Ontario and the University of Ottawa and [7]Clinical Epidemiology Program, The Ottawa Health Research Institute, Canada

Abstract

Background. Current clinical guidelines recommend that renal transplant recipients (RTRs) be classified into chronic kidney disease (CKD) stage using a creatinine-based estimate of glomerular filtration rate (GFR). However, creatinine-based equations are inaccurate in RTRs leading to frequent CKD stage misclassification. It is not known whether the classification of CKD stage would be improved using a cystatin C-based estimate of GFR.

Methods. We measured ^{99m}Tc-DTPA GFR, cystatin C and creatinine in 198 stable RTRs. GFR was estimated using cystatin C-based equations (Filler, Le Bricon and Rule) and four creatinine-based equations. We determined the proportion, overall and by CKD stage, that were classified correctly by each equation as compared to the ^{99m}Tc-DTPA GFR.

Results. The Filler equation correctly classified 76% of patients compared to only 65% with the abbreviated modification of diet in renal disease (MDRD) equation and 69% with the Cockcroft–Gault equation. In CKD stages two and four, the Filler equation correctly classified 77% and 60% of patients whereas the abbreviated MDRD equation correctly classified 46% and 93% of patients. The area under the curve by receiver operating curve analysis for overall stage classification was uniformly poor for all equations (0.52–0.56).

Conclusions. The cystatin C-based Filler and Le Bricon GFR estimates classified slightly more patients into the correct CKD stage than the standard creatinine-based equations in stable RTRs although the overall diagnostic accuracies were similar. The differences are modest and prospective studies will be needed to determine if the adoption of these equations for classification would lead to improved recognition of CKD complications or patient care.

Keywords: chronic kidney disease; creatinine; cystatin c; glomerular filtration rate; kidney transplantation

Introduction

The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation published guidelines for the diagnosis and classification of chronic kidney disease (CKD) in 2002 [1]. These guidelines recommend that patients with CKD be evaluated for the severity of renal dysfunction with a creatinine-based prediction equation [1]. The guidelines also recommend that patients be assigned to one of five stages based on the level of glomerular filtration rate (GFR). The serum creatinine concentration is a crude marker of GFR [1,2]. Various creatinine-based equations that incorporate biometric and other biochemical data have been developed in an attempt to improve the estimation of GFR[1]. These creatinine-based equations, however, are not accurate in renal transplant recipients (RTRs) [3–7] and frequently classify patients into the incorrect CKD stage [4].

The shortcomings of creatinine and creatinine-based estimates of GFR have led to the pursuit of alternate markers of GFR. Serum cystatin C has been shown to be a more sensitive marker of GFR than serum creatinine [8]. Cystatin C is a low-molecular-weight protein that functions as a cysteine protease inhibitor and is produced at a constant rate by all nucleated cells [9]. In the kidney, it is freely filtered and then

Correspondence and offprint requests to: Dr G. Knoll, Division of Nephrology, The Ottawa Hospital, Riverside Campus, 1967 Riverside Drive, Ottawa, Ontario, Canada K1H 7W9. Email: gknoll@ottawahospital.on.ca

catabolized in the proximal tubule [9]. We have recently shown that the cystatin C-based GFR estimation equations of Filler [10] and Le Bricon *et al.* [11] provide a more accurate estimate of GFR than creatinine or other cystatin C-based equations in 117 stable RTRs [3]. Recently, a novel cystatin C-based equation, derived from renal transplant recipients, has been reported [12]. It has not yet been validated in an independent sample of renal transplant recipients.

The primary objective of this study was to determine whether an estimate of GFR based on cystatin C, rather than creatinine, would result in a more accurate classification of K/DOQI CKD stage in a cohort of renal transplant recipients. A secondary objective was to assess the performance of the novel cystatin C-based Rule equation. GFR was measured with a radioisotopic reference standard and also estimated from the serum cystatin C and serum creatinine concentration using published equations. We then determined what proportion of patients was classified into the correct CKD stage with each prediction equation.

Subjects and methods

Study population

Adult RTRs with stable renal function (<30 μmol/l difference in creatinine between two most recent values) who were at least 6 months post-transplant were included in the study. The exclusion criteria were (i) inability to provide informed consent; (ii) pregnancy or breastfeeding; (iii) one or more episodes of acute rejection within preceding 3 months; (iv) life expectancy<3 months or (v) anticipated graft failure within 3 months. This analysis includes all enrolled patients with complete laboratory results as of November 04, 2005. The Ottawa Hospital Research Ethics Board approved the study. Three hundred and eight patients met the study criteria and consent was obtained from 249. Forty-eight patients withdrew from the study after consent was obtained but before any investigations were conducted, leaving 201 patients. Since only three patients with a GFR<15 ml/min/1.73 m^2 met the study criteria, stage five CKD patients were excluded from the analyses leaving 198 patients in the final cohort.

Laboratory assessment

The laboratory methods used in this study have been previously described [3]. In brief, GFR was measured by the plasma clearance of radiolabelled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) using a single injection of 10 millicuries (370 Mbq) of ^{99m}Tc-DTPA and three plasma samples at 120, 180 and 240 min post-injection [13,14]. Standard radiochemical and radiopharmaceutical purity tests on each preparation of ^{99m}Tc-DTPA revealed them to be on average 99% pure. The well counter was also verified weekly for count reproducibility. The DuBois formula [15] was used to estimate body surface area. The GFR was corrected for standard body surface area by multiplying the measured value by 1.73 and dividing by the patients' estimated body surface area. Demographic and medication data were abstracted from the medical charts on the day of enrolment into the study. Non-fasting morning blood sampling for the serum creatinine, urea, albumin and cystatin C, along with height and weight measurements, was performed at the time of ^{99m}Tc-DTPA GFR measurement.

A Beckman Coulter LX20 Pro Clinical System using manufacturer's reagents (Beckman Coulter Inc, Brea, CA, USA) and a modified Jaffe reaction were used to measure serum creatinine. The coefficient of variation for serum creatinine was 4.9% at 0.6 mg/dl (55 μmol/l), 1.7% at 1.7 mg/dl (150 μmol/l) and 1.3% at 6.8 mg/dl (600 μmol/l). A Behring BN ProSpec analyser (Dade Behring, Marburg, Germany) with an N Latex cystatin C kit (Dade Behring, Mississauga, Canada) was used to measure cystatin C. The coefficient of variation of serum cystatin C was 3.1% at 1.06 mg/l, 3.5% at 2.04 mg/l and 6.7% at 5.26 mg/l.

Calibration of the Ottawa Hospital serum creatinine to the modification of diet in renal disease (MDRD) study laboratory serum creatinine was done as recommended [16]. Fifty samples (range, 0.6 mg/dl – 4.0 mg/dl) from a variety of patient sources were sent to the Cleveland Clinic laboratory. The resulting correlation coefficient was very high (0.989) and the calibrated creatinine was calculated using the derived equation [1.076*(Ottawa Hospital serum creatinine) − 0.082]. The calibrated creatinine was used in all analyses involving the MDRD equations.

GFR was estimated with the creatinine-based MDRD, Cockcroft–Gault and Nankivell equations [1] and the cystatin C-based Le Bricon [11], Filler [10] and Rule [12] equations (Table 1). The Cockcroft–Gault and Nankivell equations, which are not expressed as ml/min/1.73 m^2, were adjusted by multiplying the value by 1.73 and dividing by the patients' body surface area as estimated by the DuBois formula [15].

Table 1. Equations to predict glomerular filtration rate using cystatin C and creatinine[a]

Reference	Equation
[b]Filler and Lepage [10]	$\text{Log (GFR)} = 1.962 + [1.123 \times \log (1/\text{cystatin C})]$
[b]Le Bricon *et al.* [11]	$\text{GFR} = [(78) \times (1/\text{cystatin C})] + 4$
[b]Rule *et al.*[12]	$\text{GFR} = 76.6 \times (\text{cystatin C})^{-1.16}$
[b,c]Original MDRD [1]	$\text{GFR} = (170) \times (\text{Cr})^{-0.999} \times (\text{age})^{-0.176} \times (\text{urea})^{-0.170} \times (\text{albumin})^{0.318} \times (0.762 \text{ if female}) \times (1.18 \text{ if black})$
[b]Abbreviated MDRD[1]	$\text{GFR} = (186) \times (\text{Cr})^{-1.154} * (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if black})$
[d]Cockcroft–Gault [1]	$\text{GFR} = [(140 - \text{age}) \times (\text{weight})]/[\text{Cr}_{\times}72] \times (0.85 \text{ if female})$
[d,e]Nankivell [1]	$\text{GFR} = [6700/(\text{Cr} \times 88.4)] + (\text{weight}/4) - (\text{urea}/2) - [100/(\text{height})^2] + 35$ (if male) or 25 (if female)

[a]Cystatin C in mg/l, serum creatinine in mg/dl.
[b]GFR, glomerular filtration rate in ml/min/1.73 m^2.
[c]MDRD, Modification of Diet in Renal Disease, urea in mg/dl, albumin in g/dl.
[d]GFR in ml/min, weight in kilograms.
[e]Urea in mmol/l, height in metres.

Analysis

CKD stage was determined according to the K/DOQI classification scheme using both the measured GFR (using ^{99m}Tc-DTPA) and the estimated GFR [1]. The proportion of patients classified into the correct K/DOQI CKD stage by each prediction equation was then determined. The proportion correctly classified was calculated for the whole study population as well as for each CKD stage separately. Receiver operating characteristic (ROC) analysis was performed to determine the diagnostic performance of each prediction equation to correctly classify patients into CKD stage. ROC analysis was performed using the MedCalc statistical package (MedCalc Software, Belgium). Finally, for each CKD stage according to the measured GFR, the proportion of patients misclassified into the other CKD stages was determined for each of the prediction equations. To complete the analysis, the bias, precision and accuracy was calculated as recommended in the National Kidney Foundation guidelines on CKD [1]. Bias was defined as the mean difference between the measured GFR (using ^{99m}Tc-DTPA) and estimated GFR (estimated GFR - measured GFR) [1]. Precision was defined as the SD of the difference between the measured and estimated GFR[1]. Accuracy was defined as the percentage of GFR estimates lying within 10% and 30% of the measured GFR [1].

Results

Table 2 shows the baseline characteristics of the cohort. Patients were predominantly Caucasian (92%). Nearly all patients (99%) were on low-dose steroids (<10 mg/day). None were on high-dose steroids. The mean measured GFR using ^{99m}Tc-DTPA was 59 ± 21 ml/min/1.73m^2. The mean GFR, serum creatinine and serum cystatin C concentrations for the cohort and for each CKD stage are reported in Table 3.

Table 4 shows the bias, precision and accuracy of the prediction equations for the whole cohort and for each CKD stage as determined by the measured (^{99m}Tc-DTPA) GFR. The Filler equation showed the least variation in bias and precision between the different CKD stages. For stages one, two and three it had at least 81% of estimates within 30% of measured GFR and in stage four, 73% of estimates were within 30% of the measured GFR. The Rule equation also had consistently high accuracy at all CKD stages except stage one where only 56% of estimates were within 30% of the measured GFR.

Classification into K/DOQI chronic kidney disease stage

Table 5 shows the proportion of estimates and their corresponding 95% confidence intervals correctly classified into K/DOQI CKD stage with each prediction equation. Overall, the cystatin C-based Filler and

Table 2. Patient characteristics

Characteristic	$n = 198$
[a]Age (yr)	53 ± 12 (19–79)
Male [*n* (%)]	128 (65)
Race [*n* (%)]	
White	182 (92)
Black	5 (3)
Asian	6 (3)
Other	5 (3)
[a]Weight (kg)	80.0 ± 17.6 (48–169)
[a]Height (cm)	167.8 ± 9.9 (142.5–200.0)
[a]Body surface area (m^2)	1.89 ± 0.23 (1.40–2.96)
Living donor [*n* (%)]	74 (37)
[a]Time post-transplant (yr)	7.2 ± 6.9 (0.6–32.9)
Primary transplant [*n* (%)]	175 (88)
Causes of renal disease [*n* (%)]	
Diabetes mellitus	26 (13)
Polycystic kidney disease	32 (16)
Glomerulonephritis	56 (28)
Hypertension	10 (5)
Other	74 (37)
Medication [*n* (%)]	
Prednisone	195 (99)
Ciclosporin	103 (52)
Tacrolimus	77 (39)
Sirolimus	6 (3)
Mycophenolate Mofetil	133 (67)
Azathioprine	35 (18)
Trimethoprim-sulfamethoxazole	37 (19)
[b]Chronic kidney disease stage [*n* (%)]	
Stage 1, GFR $\geq$ 90	16 (8)
Stage 2, GFR 60–89	79 (40)
Stage 3, GFR 30–59	88 (44)
Stage 4, GFR 15–29	15 (8)
Cockcroft-Gault eGFR	56 ± 18 (17–113)
Abbreviated MDRD eGFR	50 ± 19 (15–117)
Original MDRD eGFR	48 ± 18 (14–117)
Nankivell eGFR	59 ± 18 (14–128)
Filler eGFR	59 ± 21 (17–130)
Le Bricon eGFR	57 ± 21 (17–130)
Rule eGFR	49 ± 18 (14–110)
^{99}Tc-DTPA GFR	59 ± 21 (16–121)

[a]Data expressed as mean $\pm$ standard deviation (range).
[b]GFR in ml/min/1.73m^2 determined by ^{99m}Tc-DTPA.

Table 3. Measurements of serum creatinine, cystatin C and GFR for the study population[a]

	Creatinine (μmol/l)	Cystatin C (mg/l)	^{99m}Tc-DTPA GFR (ml/min/1.73m^2)
Cohort	148 ± 53 (60–361)	1.65 ± 0.61 (0.73–4.37)	59 ± 21 (16–121)
[b]Stage 1, GFR $\geq$ 90	95 ± 28 (51–148)	1.07 ± 0.18 (0.42–1.39)	101 ± 9 (92–121)
[b]Stage 2, GFR 60–89	112 ± 25 (64–177)	1.27 ± 0.26 (0.82–2.3)	73 ± 9 (60–89)
[b]Stage 3, GFR 30–59	164 ± 45 (79–374)	1.88 ± 0.44 (1.14–3.12)	45 ± 8 (30–59)
[b]Stage 4, GFR 15–29	260 ± 60 (143–353)	2.95 ± 0.61 (2.27–4.37)	25 ± 4 (16–29)

[a]Data expressed as mean $\pm$ standard deviation (range).
[b]GFR in ml/min/1.73m^2 determined by ^{99m}Tc-DTPA.

Table 4. Bias, precision and accuracy of creatinine and cystatin C estimates[a]

	CKD Stage	*n*	Bias	Precision	Accuracy Within	
					10%	30%
Estimates using creatinine						
Cockcroft–Gault	1	16	−18.5	15.4	19	81
	2	79	−6.1	10.9	42	92
	3	88	0.9	11.7	30	81
	4	15	4.8	8.5	20	60
	All	198	−3.2	12.9	33	84
Abbreviated MDRD	1	16	−25.7	16.6	25	50
	2	79	−11.7	11.3	28	82
	3	88	−5.6	9.3	24	80
	4	15	−1.9	8.0	27	67
	All	198	−9.4	12.2	29	80
Original MDRD	1	16	−26.2	16.5	25	44
	2	79	−12.4	11.3	22	80
	3	88	−7.5	8.6	25	77
	4	15	−4.0	6.5	33	53
	All	198	−10.7	11.6	24	74
Nankivell	1	16	−19.6	17.2	19	69
	2	79	−3.3	10.9	46	96
	3	88	4.9	9.4	39	77
	4	15	6.0	9.6	20	40
	All	198	−0.3	12.8	38	81
Estimates using cystatin C						
Filler	1	16	−13.3	15.9	31	81
	2	79	0.3	12.5	59	91
	3	88	2.1	8.5	36	90
	4	15	3.3	5.3	27	73
	All	198	0.2	11.5	44	88
Le Bricon	1	16	−22.1	12.6	12	69
	2	79	−5.1	10.1	53	95
	3	88	1.9	7.2	43	91
	4	15	6.3	4.7	20	73
	All	198	−2.5	11.2	43	89
Rule	1	16	−27.7	14.0	12	56
	2	79	−12.1	10.9	16	87
	3	88	−6.5	7.5	31	84
	4	15	−2.2	4.5	33	87
	All	198	−10.1	11.2	24	83

[a]CKD stage as determined by the measured (^{99m}Tc-DTPA) GFR; Bias was defined as the mean difference between measured (^{99m}Tc-DTPA) and estimated GFR (estimated GFR-measured GFR); precision was defined as the standard deviation of the difference between measured (^{99m}Tc-DTPA) and estimated glomerular filtration rate; both precision and bias were expressed as ml/min/1.73m^2; accuracy was defined as the proportion of values that were within 10% or 30% of the measured (^{99m}Tc-DTPA) glomerular filtration rate.

Le Bricon equations performed best, classifying 76% and 75% of patients into the correct K/DOQI CKD stage. The creatinine-based equations along with the cystatin C-based Rule equation performed less well with only 60%–70% of patients correctly classified. In addition, there was greater variation in the proportion correctly classified according to CKD stage by these equations. For example, the abbreviated MDRD equation correctly classified 84% of CKD stage three and 93% of CKD stage four patients but only 25% of CKD stage one patients. Similarly, the creatinine-based equations, Cockcroft–Gault and Nankivell, classified 83% and 82% of patients in CKD stage three but only 25% of patients in CKD stage one. The Rule equation showed the most marked differences between stages with 13%, 47%, 77% and 93% correctly classified in stages 1–4, respectively. The ROC analysis is presented in Table 6. All equations have a low area under the curve (AUC) indicating that they have limited ability to distinguish the correct CKD stage from the other possible CKD stages.

Table 7 shows the distribution of CKD stages by each prediction equation within each CKD stage as determined by the measured ^{99m}Tc-DTPA GFR. For patients in CKD stage two, 49% and 53% were misclassified as stage three when GFR was estimated using the abbreviated and original MDRD equations. The Cockcroft–Gault and Nankivell equations also showed significant underestimation of GFR in stage two CKD with 33% and 24% misclassified as stage three. In CKD stage two, the Filler equation misclassified 10% as stage one and 13% as stage three CKD. For patients with stage three CKD, the abbreviated MDRD and original equations

Table 5. Proportion of patients classified into the correct K/DOQI chronic kidney disease stage[a]

	All Stages ($n=198$)	Stage 1 ($n=16$)	Stage 2 ($n=79$)	Stage 3 ($n=88$)	Stage 4 ($n=15$)
Estimates using serum creatinine					
Cockcroft–Gault	136	4	50	73	9
%, (95% CI)	69 (62–75)	25 (10–50)	63 (52–73)	83 (74–89)	60 (36–80)
Abbreviated MDRD	128	4	36	74	14
%, (95% CI)	65 (58–71)	25 (10–50)	46 (35–57)	84 (75–90)	93 (70–99)
Original MDRD	118	2	34	70	12
%, (95% CI)	60 (53–66)	13 (4–36)	43 (33–54)	80 (70–87)	80 (55–93)
Nankivell	138	4	55	72	7
%, (95% CI)	70 (63–76)	25 (10–50)	70 (59–79)	82 (73–89)	47 (25–70)
Estimates using cystatin C					
Filler	151	7	61	74	9
%, (95% CI)	76 (70–82)	44 (23–67)	77 (67–85)	84 (75–90)	60 (36–80)
Le Bricon	148	2	61	78	7
%, (95% CI)	75 (68–80)	13 (4–36)	77 (67–85)	89 (80–94)	47 (25–70)
Rule	121	2	37	68	14
%, (95% CI)	61 (54–68)	13 (4–36)	47 (36–58)	77 (68–85)	93 (70–99)

[a]Chronic kidney disease stage as determined by the measured (^{99m}Tc-DTPA) glomerular filtration rate; data represents numbers and percent of patients classified into the correct chronic kidney disease stage.

Table 6. Area under the curve (AUC) from receiver operating characteristic analysis for chronic kidney disease stage classification

Equation	AUC (95% CI)
Cockcroft–Gault	0.54 (0.46–0.61)
Abbreviated MDRD	0.56 (0.49–0.63)
Original MDRD	0.53 (0.46–0.60)
Nankivell	0.52 (0.45–0.59)
Filler	0.56 (0.48–0.62)
Lebricon	0.55 (0.48–0.62)
Rule	0.53 (0.46–0.60)

tended to underestimate the GFR with those misclassified categorized as stage four (12% and 17%). On the contrary, the patients misclassified by the Nankivell, Le Bricon and Filler equations were mostly designated as stage two.

Discussion

This study demonstrates that the cystatin C-based prediction equations of Filler and Le Bricon, are more sensitive at classifying patients into the correct K/DOQI CKD stage than the conventional creatinine-based equations and the novel cystatin C- based Rule equation. However, no equation has superior diagnostic properties as evidenced by the uniformly poor results from the ROC analysis. The Filler equation showed a more consistent performance across all stages of CKD than the other equations. Importantly, the Filler equation correctly classified 77% of stage two CKD patients. The abbreviated and original MDRD equations were significantly worse with less than half of patients being correctly classified in stage two. Although derived from renal transplant recipients, the novel cystatin C–based Rule equation did not perform as well as the other two cystatin

Table 7. Classification of estimation equations by CKD stage[a]

CKD stage by ^{99m}Tc-DTPA GFR		1 ($n=16$)	2 ($n=79$)	3 ($n=88$)	4 ($n=15$)
CKD stage by Cockcroft-Gault equation	1	**25**	4	0	0
	2	69	**63**	11	0
	3	6	33	**83**	40
	4	0	0	6	**60**
	5	0	0	0	0
CKD stage by original MDRD equation	1	**12**	4	0	0
	2	56	**43**	1	0
	3	31	53	**80**	7
	4	0	0	17	**80**
	5	0	0	2	13
CKD stage by abbreviated MDRD equation	1	**25**	5	0	0
	2	44	**46**	2	0
	3	31	49	**84**	7
	4	0	0	12	**93**
	5	0	0	2	0
CKD stage by Nankivell Equation	1	**25**	6	0	0
	2	69	**70**	14	0
	3	6	24	**82**	47
	4	0	0	4	**47**
	5	0	0	0	6
CKD stage by Filler Equation	1	**44**	10	0	0
	2	56	**77**	11	0
	3	0	13	**84**	40
	4	0	0	4	**60**
	5	0	0	1	0
CKD stage by Le Bricon Equation	1	**12**	3	0	0
	2	88	**77**	9	0
	3	0	20	**89**	53
	4	0	0	2	**47**
	5	0	0	0	0
CKD stage by Rule Equation	1	**12**	2	0	0
	2	69	**47**	3	0
	3	19	50	**77**	0
	4	0	1	19	**93**
	5	0	0	1	7

[a]Patients were divided into K/DOQI CKD stage by measured (^{99m}Tc-DTPA) GFR. CKD stage was then determined using the creatinine and cystatin C based formulas. Data represent the proportion of patients classified in each stage. The bold values signify, the percentage of patients in a given measured (^{99m}Tc-DTPA) CKD stage correctly classified by the prediction equation.

C-based equations in our population. Particularly, the Rule equation had a very high negative bias in CKD Stages one and two (-27 ml/min/1.73 m^2 and -12 ml/min/1.73 m^2). The reason for this remains unclear and warrants further study. Differences in reference study measures (renal iothalamate clearance *vs* plasma ^{99m}Tc-DTPA clearance) are unlikely to account for these findings [17]. Further evaluation of the performance of these novel equations is warranted in populations distinct from the derivation sample.

Our findings are similar to a recent report by Mariat *et al*. [4] which examined the performance of the creatinine-based prediction equations (Cockcroft–Gault and MDRD) in 284 renal transplant recipients. They found that the abbreviated MDRD equation classified 63% of patients into the correct K/DOQI CKD stage, which is similar to the value of 65% in our current study. They concluded that the K/DOQI guidelines could be flawed with respect to transplantation and that the original recommendations regarding GFR evaluation be revised [4].

The K/DOQI CKD classification scheme based on GFR was designed in part to highlight what complications should be anticipated, investigated and treated in patients with CKD [1]. The use of this classification scheme is justified on the basis that complications of renal failure correlate with stage of CKD [1]. Karthikeyan *et al*. [18] have demonstrated a high prevalence of CKD complications such as anaemia, hypocalcaemia, hyperphosphataemia, hypertension, metabolic acidosis and hypoalbuminaemia in a large cohort of RTRs The prevalence of these complications increased significantly with CKD stage as determined by the abbreviated MDRD GFR. Despite this, only 27% of patients with significant anaemia were on erythropoietin therapy [18]. In addition, the majority of hyperphosphataemic and hypocalcaemic patients were not on phosphate binders or calcium supplements. These findings suggest that CKD complications are not always appropriately identified in renal transplant recipients. In this study, the greatest value of the Filler equation over the creatinine-based equations was seen in CKD stage two. Since CKD complications are less prevalent in Stage two [18], it is unlikely that more CKD complications would be recognized with the use of a cystatin C-based GFR estimate. However, classification using cystatin C would lead to less false positive labelling of transplant recipients as having CKD. This may result in more efficient clinical care if management guidelines based on CKD stage are strictly followed [1].

There are conflicting reports in the literature regarding the direction and degree of bias of the creatinine-based equations in renal transplant recipients. In some studies, the MDRD equation underestimates GFR [3,12,19] leading to a negative bias, while in others, it over-estimates GFR [4–6] leading to a positive bias. The reasons for these differences are likely multi-factorial. First, with the exception of one other study [6], serum creatinine was not calibrated to the MDRD study laboratory. Differences between laboratories in creatinine calibration can have profound effects on GFR estimation [16]. Second, the GFR spectrum varied between studies. We and others, have demonstrated significant differences in equation performance at different levels of GFR, with greater negative bias and worse precision for the MDRD equation at higher GFR [3,4]. Almost half of our cohort had GFR measures >60 ml/min/1.73 m^2. It is therefore not surprising that we found an overall large negative bias for the MDRD equation. The inclusion of significant numbers of patients with relatively well-preserved GFR likely reflects the care taken during recruitment to ensure that patients with lower creatinine values were enrolled. Finally, differing methodologies were used to measure GFR, which likely contributed to some of the noted differences between equation performance.

In contrast to our findings, Poge *et al*. [5] have recently shown that the bias and accuracy of the abbreviated MDRD and Filler equations were similar in a cohort of renal transplant recipients. The mean GFR in this cohort was 39.5 ml/min/1.73 m^2, which was significantly lower than in our study and may explain the discrepant findings. Unfortunately, they do not report how well each equation classified patients into CKD stage, which prevents a direct comparison with our findings.

The strengths of this study include the measurement of cystatin C, serum creatinine and ^{99m}Tc-DTPA GFR on the same day. As well, all serum creatinine values were measured in the same laboratory and were calibrated to the MDRD study laboratory, as recommended for the evaluation of the MDRD equations [1,16]. However, limitations to the study should be noted. First, the population is largely Caucasian. Although cystatin C levels appear to be independent of race [9], firm conclusions regarding the classification of CKD stage in non-Caucasian populations cannot be made from our data. Second, we did not measure thyroid function, which is known to affect cystatin C independently of changes in GFR [9]. It is, however, improbable that there would be substantial unrecognized thyroid dysfunction in this group of patients who receive regular medical care. Third, we only measured cystatin C once in each patient. There is conflicting data about the intra-patient variability of cystatin C [9]. However, recent evidence suggests that intra-patient variability is lower for cystatin C than for creatinine [20]. Fourth, there were small numbers of patients with CKD stages one and four, limiting our ability to conclusively distinguish differences in classification ability between equations in these stages. Fifth, almost all of our patients were on low-dose steroids (<10 g/day). There is mounting evidence that low-dose steroids does lead to an increase in cystatin C levels independently of GFR [9,21]. It is relevant to note that, despite a potential confounding effect of low-dose steroids on the cystatin C level, the cystatin C-based equations of Filler and Le Bricon still underestimated GFR to a lesser degree than the creatinine-based equations. Finally, 37 patients were

on trimethoprim-sulfamethoxasole, which could affect the performance of the creatinine-based equations. We determined the bias, precision and accuracy of the creatinine-based equations in patients receiving and not receiving trimethoprim-sulfamethoxasole (data not shown). Those on trimethoprim-sulfamethoxasole demonstrated greater biases than those not on trimethoprim-sulfamethoxasole. The effect of trimethoprim-sulfamethoxasole on equation performance was greatest for the abbreviated and original MDRD equations with 30% accuracy of only 62% and 60%, respectively. This is perhaps not surprising given that these equations were developed in patients who were not prescribed trimethoprim-sulfamethoxasole. Nevertheless, the accuracy of the Filler and Lebricon equations remained superior to the creatinine-based equations in the cohort not on trimethoprim-sulfamethoxasole.

GFR was measured using the plasma clearance of ^{99m}Tc-DTPA. To our knowledge, there is no published data comparing the performance of the various available GFR markers (inulin, iothalamate, ^{99m}Tc-DTPA) and clearance techniques (plasma and renal) in renal transplant recipients. In the non-renal transplant population, comparative studies are limited by small sample sizes, non-standardized plasma collection protocols and the reliance on spontaneously voided urine collections. ^{125}I-Iothalamate and ^{99m}Tc-DTPA are the most commonly used GFR markers in both clinical practice and experimental protocols. Morton *et al*. [17] reported an excellent correlation between renal ^{125}I-Iothalamate clearance and plasma ^{99m}Tc-DTPA clearance ($r = 0.966$) with no significant difference between pairs of GFR values in 18 patients with GFR values greater than 20 ml/min. Rehling *et al*.[13] have demonstrated an equally robust correlation ($r = 0.97$) between plasma clearance of ^{99m}Tc-DTPA and renal clearance of inulin in a study of 20 patients with CKD. On average, plasma ^{99m}Tc-DTPA clearance overestimated inulin renal clearance by only 3.5 ml/min. However, no patient had a measured inulin GFR >80 ml/min. It is possible that the accuracy of the ^{99m}Tc-DTPA GFR is lower at higher GFRs. The absence of evidence supporting this putative overestimation precludes any estimation of the effect on study results. GFR measurement techniques are awkward, expensive and time consuming. The simultaneous measurement of GFR using two or more techniques is generally too cumbersome for routine study protocols. There is however, a pressing need for high quality studies exploring the differences between the various GFR measurement techniques across the spectrum of GFR.

In conclusion, this analysis has shown that the estimated GFR based on cystatin C does improve the classification of RTRs into the correct K/DOQI CKD stage. However, the improved classification appears modest and is limited to earlier stages of CKD where complications are less prevalent [18]. Furthermore, cystatin C is considerably more expensive and less available than serum creatinine. Until, prospective studies demonstrate that the adoption of these equations for classification leads to improved recognition of CKD complications or patient care, there is likely no benefit of their use for the purpose of classification and CKD staging in routine clinical care of renal transplant recipients. Classification using cystatin C equations may, however, lead to less misclassification at the population level in studies examining CKD in transplantation. The improved accuracy and classification ability of the Filler and Lebricon equations at higher levels of GFR needs further study in non-transplant patients where CKD stage misclassification by the creatinine-based equations is a significant problem.

Acknowledgements. This study was funded by The Physicians' Services Incorporated Foundation (Grant # R03-59) and Astellas Pharma Canada. Instrumentation and reagents to measure cystatin C were provided by Dade Behring. We thank the staff and patients from the renal transplant program that participated in the study. We wish to acknowledge Alan Thibeau, Nur Jamal, Ian Graham, Lisa Banfield, Sheila Dowell, Philip St. Laurent, Julie Noel, Elyse Bienvenue, Martine Blouin, Claudine Messier and Sunil Thakrar for assistance with the DTPA GFR measurements; Marcella Cheng-Fitzpatrick and Anna Micucci for data management; and, Zeyad Alrayes, Judy Cheesman, Darlene Hackett, Margo McCoshen, Paul McLoughlin, Amy Pocock, Lisa South and Jennifer Bowes for their invaluable assistance in conducting this study.

Conflict of interest statement: None declared.

References

1. National Kidney Foundation.K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease:Evaluation, Classification and Stratification. *American Journal of Kidney Diseases* 2003; 39: S1–S266
2. Levey AS. Measurement of renal function in chronic renal disease. [Review] [129 refs]. *Kidney Int* 1990; 38: 167–184
3. White C, Akbari A, Hussain N *et al*. Estimating Glomerular Filtration Rate in Kidney Transplantation: A Comparison between Serum Creatinine and Cystatin C-Based Methods. *J Am Soc Nephrol* 2005; 16: 3763–3770
4. Mariat C, Alamartine E, Afiani A *et al*. Predicting glomerular filtration rate in kidney transplantation: are the K/DOQI guidelines applicable? *Am J Transplant* 2005; 5: 2698–2703
5. Poge U, Gerhardt T, Stoffel-Wagner B *et al*. Cystatin C-based calculation of glomerular filtration rate in kidney transplant recipients. *Kidney Int* 2006; 70: 204–210
6. Poggio ED, Wang X, Weinstein DM *et al*. Assessing glomerular filtration rate by estimation equations in kidney transplant recipients. *Am J Transplant* 2006; 6: 100–108
7. Risch L, Huber AR. Assessing glomerular filtration rate in renal transplant recipients by estimates derived from serum measurements of creatinine and cystatin C. *Clinica Chimica Acta* 2005; 356: 204–211
8. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; 40: 221–226
9. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. [Review] [515 refs]. *Crit Rev Cl Lab Sci* 2004; 41: 467–550
10. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? *Pediatr Nephrol* 2003; 18: 981–985

11. Le Bricon T, Thervet E, Froissart M *et al*. Plasma cystatin C is superior to 24-h creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation. [comment] *Clin Chem* 2000; 46: 1206–1207
12. Rule AD, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by cystatin C among different clinical presentations. *Kidney Int* 2006; 69: 399–405
13. Rehling M, Moller ML, Thamdrup B, Lund JO, Trap-Jensen J. Simultaneous measurement of renal clearance and plasma clearance of 99mTc-labelled diethylenetriaminepenta-acetate, 51Cr-labelled ethylenediaminetetra-acetate and inulin in man. *Clin Sci* 1984; 66: 613–619
14. Russell CD. Optimum sample times for single-injection, multisample renal clearance methods. *J Nuc Med* 1993; 34: 1761–1765
15. DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. *Arch Inten Med* 1916; 17: 863–871
16. Coresh J, Astor BC, McQuillan G *et al*. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002; 39: 920–929
17. Morton KA, Pisani DE, Whiting JH,Jr., Cheung AK, Arias JM, Valdivia S. Determination of glomerular filtration rate using technetium-99m-DTPA with differing degrees of renal function. *J Nucr Med Tech* 1997; 25: 110–114
18. Karthikeyan V, Karpinski J, Nair RC, Knoll G. The burden of chronic kidney disease in renal transplant recipients. *Am J Transplant* 2004; 4: 262–269
19. Mariat C, Alamartine E, Barthelemy J-C *et al*. Assessing renal graft function in clinical trials: Can tests predicting glomerular filtration rate substitute for a reference method? *Kidney Int* 2004; 65: 289–297
20. Podracka L, Feber J, Lepage N, Filler G. Intra-individual variation of cystatin C and creatinine in pediatric solid organ transplant recipients. *Pediatric Transplant* 2005; 9: 28–32
21. Poge U, Gerhardt TM, Stoffel-Wagner B *et al*. Beta-Trace protein is an alternative marker for glomerular filtration rate in renal transplantation patients. [see comment] *Clin Chem* 2005; 51: 1531–1533

Received for publication: 10.10.06
Accepted in revised form: 27.4.07