Comparison of creatinine and cystatin C based eGFR in the estimation of glomerular filtration rate in Indigenous Australians: The eGFR Study

Running title: Cystatin C and eGFR

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Abstract

Background: The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that combines creatinine and cystatin C is superior to equations that include either measure alone in estimating glomerular filtration rate (GFR). However, whether cystatin C can provide any additional benefits in estimating GFR for Indigenous Australians, a population at high risk of end-stage kidney disease (ESKD) is unknown.

Methods: Using a cross-sectional analysis from the eGFR Study of 654 Indigenous Australians at high risk of ESKD, eGFR was calculated using the CKD-EPI equations for serum creatinine (eGFRcr), cystatin C (eGFRcysC) and combined creatinine and cystatin C (eGFRcysC+cr). Reference GFR (mGFR) was determined using a non-isotopic iohexol plasma disappearance technique over 4 hours. Performance of each equation to mGFR was assessed by calculating bias, % bias, precision and accuracy for the total population, and according to age, sex, kidney disease, diabetes, obesity and c-reactive protein.

Results: Data were available for 542 participants (38% men, mean [sd] age 45 [14] years). Bias was significantly greater for eGFRcysC (15.0 mL/min/1.73m^2; 95%CI 13.3-16.4, p<0.001) and eGFRcysC+cr (10.3; 8.8-11.5, p<0.001) compared to eGFRcr (5.4; 3.0-7.2). Accuracy was lower for eGFRcysC (80.3%; 76.7-83.5, p <0.001) but not for eGFRcysC+cr (91.9; 89.3-94.0, p=0.29) compared to eGFRcr (90.0; 87.2-92.4). Precision was comparable for all equations. The performance of eGFRcysC deteriorated across increasing levels of c-reactive protein.

Conclusion: Cystatin C based eGFR equations may not perform well in populations with high levels of chronic inflammation. CKD-EPI eGFR based on serum creatinine remains the preferred equation in Indigenous Australians.

Words: 248 (max 250)

Keywords: CKD-EPI equation, creatinine, Cystatin C, GFR, Indigenous

Short Summary (3-4 sentences pointing out the main message):

Accurate estimation of renal function in Indigenous Australians is vital to identifying patients for clinical management as this population is at high risk of end-stage kidney disease. Our findings indicate that the CKD-EPI eGFR equation based on serum creatinine is the preferred equation in this population. Furthermore, cystatin C based eGFR may not be the optimum equation for estimating GFR in populations with a heavy burden of chronic inflammation.

1.0 Introduction

Aboriginal and Torres Strait Islander Australians are the Indigenous people of Australia and experience disproportionately high rates of chronic kidney disease (CKD) compared to non-Indigenous Australians. CKD leads to devastating health and social burdens as it progresses to end-stage kidney disease (ESKD), and is associated with high rates of cardiovascular disease, diabetes and premature mortality in this population [1]. Early detection of reduced kidney function is important, as early-stage kidney disease is often asymptomatic yet is associated with increased morbidity and mortality [2].

Current international guidelines recommend the estimation of glomerular filtration rate (GFR) and measurement of urine albumin to creatinine ratio for the detection and assessment of CKD. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on serum creatinine, age, gender and ethnicity is recommended and widely used in clinical practice [2]. However, serum creatinine-based eGFR may not be the optimal kidney filtration marker in some populations, as it can be affected by non-GFR factors, in particular, low muscle mass [3]. Cystatin C, a small molecular weight protein, is an alternative filtration marker that is also freely filtered through the glomerulus, with production less affected by muscle mass [4]. The CKD-EPI equation that combines both serum creatinine and cystatin C has been shown to be more accurate than equations that included either creatinine or cystatin C alone [5]. As such, the Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that eGFR based on creatinine and cystatin C be used as a confirmatory test in certain clinical situations where estimation of GFR with serum creatinine alone may be inaccurate [2].

Indigenous Australians have a "linear" body build (narrow shoulders and hips, long limbs and short torso), which is proportionally associated with less muscle mass for a given weight [6, 7]. Though previous analysis of our data supports the use of CKD-EPI based on creatinine in Indigenous Australian populations [8, 9], we have demonstrated that misclassification of GFR by creatinine based CKD-EPI eGFR was greatest at low weights (<72.5 kg) and that the inclusion of weight into the equation mitigated this difference. However, the strength of the association between the estimating equation based on serum creatinine and measured GFR was not substantially improved with the addition of other anthropometric measures, including fat free mass [10]. Equations combining cystatin C and creatinine perform well in multi-

ethnic Asian populations also comprising individuals with relatively lower body mass indices [11], and in older adults with lower body mass indices [12].

It is not known whether cystatin C can provide any benefits in estimating GFR for Indigenous Australians. Therefore, the aim of this study was to compare the performance of CKD-EPI eGFR equations based on serum creatinine (eGFRcr) to those based on either cystatin C (eGFRcysC) or a combination of cystatin C and creatinine (eGFRcysC+cr) in predicting reference GFR in Indigenous Australians.

2.0 Materials and Methods

2.1 Participants

The methods have been described previously [13]. Participants were Aboriginal and/or Torres Strait Islander Australians, and men and women aged ≥16 years were recruited between 2007 and 2011 from urban, rural and remote centres (within the Northern Territory, Queensland and Western Australia) across five pre-defined strata: (i) "healthy" people without diabetes, CKD or albuminuria, (ii) participants with diabetes or albuminuria and eGFR (MDRD-4) >90 mL/min/1.73 m^2; (iii) eGFR 60–90 mL/min/1.73 m^2; (iv) eGFR30–59 mL/min/1.73 m^2; (v) eGFR < 15–29 mL/min/1.73 m^2. Participants with CKD and/or diabetes were recruited from participating medical services, and the "healthy" group were identified through community networks and staff of participating medical facilities. Individuals were not eligible if they were identified as having rapidly changing kidney function, receiving dialysis, pregnant or breastfeeding, or had an allergy or adverse reaction to iodine-based contrast media. At baseline, 654 Indigenous participants were recruited to the study. This analysis was based on 542 participants. We excluded participants from this analysis if they were (i) acutely unwell (n=1); (ii) had a urinary tract infection (n=7); (iii) were aged <18 years (n=13), (iv) did not have an enzymatic creatinine measure (n=10), or (iv) did not have a cystatin C measure (n=81). Compared to participants who were missing measurements for creatinine or cystatin C, participants who were included were younger (mean [sd] 45 [0.6] *vs.* 48 (1.6) years), less likely to be women (62 *vs.* 67%), less likely to have diabetes (38 *vs.* 48%) and macroalbuminuria (19 vs. 36%), but had similar mean BMI and blood pressure (data not shown). Participants provided informed consent, and the Human Research Ethics Committees of the joint Menzies School of Health Research—Northern Territory Department of Health Human Research Ethics Committee, including the Aboriginal subcommittee; Central

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Australian Human Research Ethics Committee; Western Australian Aboriginal Health Information and Ethics Committee, Royal Perth Measurements Hospital Ethics Committee and Cairns and Hinterland Health Services District Human Research Ethics Committee approved the study.

2.2 Measurements

Participants underwent a health examination which included performance of the reference measure of GFR, collection of urine and non-fasting venous blood samples, anthropometric measurements and the administration of questionnaires [13]. Reference GFR was determined by measuring the renal clearance of non-isotopic iohexol (300 mg/mL, Omnipaque; GE Healthcare, Rydalmere, NSW, Australia) over 4 hours (with measurements at 120, 180 and 240 minutes after the injection) using previously described methods [13]. Venous blood samples collected at 120 minutes after the injection were also used to measure serum creatinine and cystatin C. Venous blood samples were refrigerated, centrifuged within 4 hrs and aliquoted for transportation on ice prior to storage at –80 °C freezer. Iohexol was measured at a central laboratory (Austin Health, Melbourne Australia) using a validated HPLC assay modified from Niculescu-Duvaz et al. [14], and reference GFR (mL/min/1.73 m^2) was calculated [14, 15].

Serum creatinine and cystatin C were measured from thawed frozen sera by a single laboratory (Melbourne Pathology, Melbourne Australia). Serum creatinine was measured using a Roche IDMS- aligned enzymatic method (Roche Diagnostics, Australia) and cystatin C measured using an immunoturbidimetric assay standardised according to the IFCC (Roche Diagnostics, Australia) [16]. Cystatin C remains stable over several freeze-thaw cycles [17]. We calculated eGFR based on serum creatinine, cystatin C or a combination of both measures using the CKD-EPI equations [5].

Random urine samples were collected as part of standard clinical care to measure albumin and creatinine (to determine urine albumin to creatinine ratio) by local accredited pathology providers located at each of the recruitment sites using methods that have been previously reported [13]. Height was measured to the nearest 0.1 cm using a stadiometer on two occasions and a third measure was recorded if the first two measures differed by more than 0.5cm. Weight to the nearest 0.1 kg was measured twice using digital scales and a third measure taken if the first two differed (Seca Model 767 and 841, Seca Deutschland,

Hamburg, Germany). Waist and hip circumferences were measured alternatively to the nearest 0.1 cm at least twice, and a third measure taken if the first two differed by more than 1.0 cm. Waist circumference was measured at the midway point between the iliac crest and the costal margin, and hip circumference over the widest part of the buttocks. Fat free mass (FFM) was measured using single frequency bioimpedance (ImpediMed, USA) in a sub-group of n=483.

C-reactive protein (using high sensitivity assays) and haemoglobin A_{1c} (HbA$_{1c}$) were measured by local accredited pathology providers [13]. Diabetes was defined as a self-reported diagnosis of Type 2 diabetes or HbA$_{1c}$ ≥6.5% [18]. Self-reported cigarette smoking status was also recorded as current, ex-smoker and never smoked.

2.3 Statistical analysis

Participant characteristics were described in terms of means (sd) or medians (inter-quartile range) for continuous variables, and numbers (proportions) for categorical variables, for the whole study population, and according to reference GFR groups: <60, 60-89 and ≥90 mL/min/1.73m^2). Performance of CKD-EPI equations based on serum creatinine (eGFRcr), cystatin C (eGFRcysC) and cystatin C plus serum creatinine (eGFRcysC+cr) to reference GFR were assessed for the whole study population, then according to sex, age groups (18-40, 40-55 and ≥55 years), ethnicity (Aboriginal and/or Torres Strait Island), body mass index (BMI) based on World Health Organisation categories for Asian populations to account for the potentially different associations between BMI and risk of chronic conditions in this population (<23, 23-27.5 and ≥27.5 kg/m^2 - the lowest categories were combined as there were too few participants with a BMI <18.5kg/m^2) [19], waist circumference (<80cm women and <90 cm for men vs >80 cm women and >90 cm men), c-reactive protein (<3, 3-10 and >10 mg/l) sex, ethnic specific tertiles of % FFM and diabetes (diabetes vs no diabetes).

We used metrics that have been previously reported for assessing equation performance [5, 20, 21]. Bias was defined as the median difference (mL/min/1.73m^2) between the reference GFR and the estimated GFR (i.e. mGFR– eGFR), and percentage bias as the median percentage difference relative to mGFR. Accuracy was defined as the percentage of eGFR values that fell within 30% of their corresponding mGFR value, and precision as the interquartile range of the absolute differences. Confidence intervals were calculated using the binomial exact method for proportions. We tested the difference between eGFR equations for

each of the performance measures using the Kruskal Wallis test to compare median absolute bias and median absolute percentage bias, quantile regression to compare precision and McNemar's test to compare accuracy. In order to compare estimated values across categories of c-reactive protein we calculated p-values using the Mann-Whitney U-test for bias and % bias, interquantile range regression for precision and χ^2-test for accuracy. Individual differences between reference GFR and each of the eGFR equations were plotted, and quantile regression using the qreg command in Stata v14.1 was used to examine how bias varied as a (cubic) function of GFR. The function has been plotted across the whole range of eGFR. Analyses were conducted in Stata 14.1 (StataCorp, College Station, TX, USA).

3.0 Results

3.1 Participant characteristics

Table 1 outlines the characteristics of the study population which comprised 542 (38% men) Indigenous Australians with a mean (sd) age of 45 (14) years. The majority of participants were Aboriginal (70%), 21% were Torres Strait Islander Australians and 9% were Aboriginal and Torres Strait Islander Australians. The median (IQR: inter-quartile range) of mGFR for the study population was 104 (83-122) mL/min/1.73m^2, and 101 (81-114) mL/min/1.73m^2 for eGFRcr, 89 (69-104) mL/min/1.73m^2 for eGFRcysC, and 96 (76-108) mL/min/1.73m^2 for eGFRcysC+cr. Decreasing mGFR ($\geq$90, 60-89, <60 mL/min/1.73m^2) was associated with older age, lower weight, greater HbA$_{1c}$, slightly lower BMI, but larger waist circumference and waist to hip ratio, lower percent of FFM, and lower levels of c-reactive protein. Participants with lower mGFR were also less likely to be smokers, and more likely to have diabetes or albuminuria.

Table 1. Participant characteristics for the overall study population and according to reference GFR groups: the eGFR study.

	All Participants	mGFR < 60 mL/min/1.73m²	mGFR 60-89 mL/min/1.72m²	mGFR ≥90 mL/min/1.73 m²
	n=542	n=63	n=111	n=368
Age (years)	45 (14)	59 (11)	53 (13)	39 (12)
Men (%)	205 (38)	20 (32)	42 (38)	143 (39)
Height (cm)	167 (8)	163 (7)	166 (8)	167 (8)
Weight (kg)	83 (21)	78 (21)	81 (23)	85 (21)
BMI (kg/m²)	30.0 (7.2)	29.1 (7.6)	29.2 (7.7)	30.4 (7.0)
Percent Fat Free Mass	64.4 (9.3)	64.9 (9.3)	66.0 (9.6)	65.6 (9.5)
C-reactive protein (mg/L)	5.8 (3-11)	5.0 (2.6-10)	5.2 (2.1-10)	6.0 (3-11)
Waist (cm)	100 (16)	102 (16)	100 (17)	100 (16)
Waist-hip ratio	0.94 (0.09)	0.98 (0.09)	0.94 (0.10)	0.93 (0.09)
Current smoker (%)	226 (42)	15 (24)	33 (30)	178 (49)
Diabetes (%)	216 (40)	42 (68)	52 (47)	122 (33)
Haemoglobin A_{1c} (mmol/L)	49.2 (19.0)	54.7 (18.2)	51.1 (21.0)	47.8 (18.4)
Microalbuminuria (%)[a]	103 (20)	12 (20)	23 (23)	68 (19)
Macroalbuminuria (%)[a]	98 (19)	41 (69)	25 (25)	32 (9)
Enzymatic creatinine (nmol/L)	71 (57-87)	142 (103-194)	76 (67-94)	64 (54-77)
Cystatin (mg/L)	0.92 (0.82-1.10)	1.95 (1.58-2.65)	1.11 (0.97-1.24)	0.87 (0.79-0.95)
CKD-EPI eGFRcr (mL/min/1.73m²)	101 (81-114)	39 (27-52)	81 (71-94)	109 (97-118)
CKD-EPI eGFRcysC (mL/min/1.73m²)	89 (69-104)	31 (20-41)	68 (58-79)	99 (87-109)
CKD-EPI eGFRcysC+cr (mL/min/1.73m²)	96 (76-108)	34 (23-44)	74 (63-85)	104 (94-113)
Reference GFR (mL/min/1.73m²)	104 (83-122)	43 (27-53)	80 (72-86)	115 (103-127)

Data are number (%) for categorical variables and mean (standard deviation) or median (inter-quartile range) for continuous variables.

[a] Microalbuminuria 27-265 mg/g (3-30 mg/mmol); Macroalbuminuria > 265 mg/g (30mg/mmol).

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFRcr: CKD-EPI estimated glomerular filtration rate equation based on serum creatinine; eGFRcysC: CKD-EPI estimated glomerular filtration rate equation based on cystatin C; eGFRcysC+cr: CKD-EPI estimated glomerular filtration rate equation based on cystatin C and serum creatinine; mGFR: Glomerular Filtration Rate measured using non-isotopic iohexol.

3.2 Performance characteristics of eGFRcysC and eGFRcysC+cr compared to eGFRcr with reference to reference GFR

The performance of CKD-EPI eGFR equations with reference to mGFR is presented in Table 2. For the whole study population, bias was significantly greater for eGFRcysC (15.0mL/min/1.73m²; 95%CI 13.3-16.4, p<0.001) and eGFRcysC+cr (10.3; 8.8-11.5, p<0.001) compared to eGFRcr (5.4; 3.0-7.2). Accuracy was significantly lower for eGFRcysC (80.3%; 76.7-83.5, p <0.001) but not for eGFRcysC+cr (91.9; 89.3-94.0, p=0.29)

compared to eGFRcr (90.0; 87.2-92.4). Precision was comparable for all eGFR equations under examination.

Table 2. Performance characteristics of CKD-Epi eGFRcr, eGFRcysC and eGFRcysC+cr to reference GFR in Indigenous Australians, for the overall study populations and according to demographic and risk factor characteristics: the eGFR study.

	n	Bias eGFRcr	Bias eGFRcysC	Bias eGFRcysC+cr	% Bias eGFRcr	% Bias eGFRcysC	% Bias eGFRcysC+cr	Precision eGFRcr	Precision eGFRcysC	Precision eGFRcysC+cr	Accuracy eGFRcr	Accuracy eGFRcysC	Accuracy eGFRcysC+cr
Total population	542	5.4	15.0	10.3	5.8	16.6	11.5	20.2	21.0	18.0	90.0	80.3	91.9
		(3.0-7.2)	(13.3-16.4)**	(8.8-11.5)**	(3.5-7.6)	(15.2-18.3)**	(9.9-13.0)**	(-5.0-15)	(5.7-26.7)	(1.8-19.8)	(87.2-92.4)	(76.7-83.5)**	(89.3-94.0)
Men	205	8.4	14.9	12.6	8.4	15.5	13.0	19.9	22.7	19.0	87.8	81.0	90.7
		(6.5-11.4)	(12.2-16.6)**	(9.9-13.9)**	(7.0-11.0)	(12.9-18.5)**	(10.9-16.0)*	(-0.85-19.1)	(5.2-27.9)	(3.1-22.1)	(82.5-92.0)	(74.9-86.1)	(85.9-94.3)
Women	337	2.5	15.2	9.2	2.7	17.0	10.6	20.1	20.3	18.1	91.4	79.8	92.6
		(0.2-5.4)	(13.2-16.9)**	(7.5-10.9)**	(0.2-5.7)	(15.6-19.2)**	(8.9-12.2)**	(-5.0 to 13.2)	(6.2-26.4)	(0.45-18.5)	(87.9-94.2)	(75.1-84.0)**	(89.2-95.1)
Age 18-40 years	215	1.7	11.7	7.7	1.5	11.4	6.8	23.1	22.4	21.0	93.0	91.2	97.7
		(-1.1 to 4.5)	(9.9-15.0)**	(5.9-9.6)**	(-0.9 to 4.1)	(9.1-12.9)**	(5.1-9.0)**	(-7.9-15.1)	(2.3-24.8)	(-1.3 to 19.7)	(88.8-96.0)	(86.5-94.6)	(94.7-99.2)*
Age 40-55 years	189	9.6	18.2	14.1	10.1	19.9	15.0	19.1	24.4	21.3	87.8	73.0	87.8
		(7.5-12.3)	(15.2-21.3)**	(11.6-16.3)**	(8.0-11.3)**	(16.9-21.8)**	(12.1-17.5)**	(-0.1 to 19.0)	(9.0-33.4)	(4.3-25.6)	(82.3-92.1)	(66.1-79.2)**	(82.3-92.1)
Age ≥55 years	138	1.6	14.7	9.1	2.9	22.7	14.0	16.6	16.2	11.7	88.4	73.2	88.4
		(-0.6 to 5.2)	(12.5-17.0)**	(6.8-11.0)**	(-1.0 to 9.3)	(19.2-25.1)**	(11.6-16.8)**	(-4.5 to 12.1)	(7.2-23.4)	(3.9-15.7)	(81.9-93.2)	(65.0-80.4)**	(81.9-93.2)
eGFRcr <60 mL/min/1.73m²	69	5.3	11.3	9.9	13.7	25.2	22.8	11.1	11.3	8.8	76.8	59.4	71.0
		(3.7-8.3)	(7.8-13.6)**	(7.4-11.0)*	(10.1-20.9)	(20.2-30.6)*	(18.2-27.1)*	(0.8-12.0)	(5.7-17.0)	(5.1-14.0)	(65.1-86.1)	(58.8-81.3)	(58.8-81.3)*
eGFRcr 60-90 mL/min/1.73m²	114	8.3	13.1	10.8	9.8	16.7	12.8	21.2	18.3	15.3	85.1	83.3	94.2
		(3.7-12.6)	(10.7-16.1)*	(8.2-12.9)	(4.3-13.3)	(12.8-20.7)*	(10.0-15.3)*	(-3.1 to 18.1)	(4.1-22.4)	(3.8-19.1)*	(77.2-91.1)	(75.2-89.7)	(91.2-96.3)
eGFRcr ≥90 mL/min/1.73m²	359	3.2	12.4	9.4	3.0	11.0	8.1	21.6	24.3	21.6	94.2	83.3	96.4
		(1.6-6.6)	(10.4-15.3)**	(7.4-12.2)**	(1.4-5.8)	(9.3-12.6)**	(6.6-9.9)**	(-6.3 to 15.2)	(6.1-30.5)	(0.40-22.0)	(91.2-96.3)	(79.0-87.0)**	(93.9-98.1)
BMI <23 kg/m²	67	-5.8	6.01	1.2	-6.6	7.0	1.4	17.2	15.9	13.0	85.1	94.0	97.0
		(-7.9 to -2.0)	(3.4-9.3)*	(-2.0 to 4.7)	(-9.5 to -3.2)	(4.1-11.5)	(-2.4 to 4.9)	(-14.1 to 3.0)	(-2.8 to 13.1)	(-6.3 to 6.7)	(74.3-92.6)	(85.4-98.3)	(89.6-99.6)*
BMI 23-27.5 kg/m²	106	6.5	13.7	9.6	6.8	14.2	9.5	16.0	15.6	14.8	89.6	83.0	87.7
		(1.4-8.5)	(10.6-16.0)**	(7.1-11.3)*	(1.7-9.2)	(11.0-17.2)**	(7.6-13.5)*	(-3.2 to 12.8)	(5.2-20.8)	(1.9-16.6)	(82.2-94.7)	(74.5-89.6)	(79.9-93.3)
BMI ≥27.5 kg/m²	205	8.3	22.4	14.8	7.9	23.6	16.0	21.4	23.4	18.9	89.8	69.8	89.8
		(5.4-9.5)	(17.3-26.2)**	(13.1-17.2)**	(5.5-10.0)	(20.8-25.8)	(13.9-17.6)**	(-2.8 to 18.7)**	(11.1-34.5)	(6.6-25.5)	(84.8-93.5)	(63.0-76.0)**	(84.8-93.5)
No diabetes	325	3.6	12.6	8.2	3.5	12.8	8.2	20.3	20.3	17.0	92.6	89.8	96.0
		(1.5-6.9)	(10.6-14.8)	(7.0-10.1)	(1.5-7.0)	(11.0-14.0)	(7.0-10.0)	(-5.9 to 14.4)	(2.4-22.8)	(0.07-17.1)	(89.2-95.2)	(86.0-92.9)	(93.3-97.9)
Diabetes	216	7.0	18.3	13.1	8.2	23.4	16.6	22.5	22.2	20.2	86.6	66.3	86.1
		(4.8-8.6)	(16.2-22.2)	(11.1-16.5)	(5.2-11.2)	(21.3-25.9)	(13.9-19.3)	(-3.0 to 19.5)	(9.8-32.0)	(5.1-25.3)	(81.2-90.8)	(59.5-72.5)	(80.8-90.4)

P values were calculated to compare eGFR equations based on cystatin C to eGFR based on serum creatinine;* p <0.05; ** p <0.001

Bias and % Bias are median (95% CI); Precision is interquartile range of the bias (25th, 75th percentile of the bias); Accuracy is % of eGFR within 30% of reference GFR (95% CI).

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFRcr: CKD-EPI estimated glomerular filtration rate equation based on serum creatinine; eGFRcysC: CKD-EPI estimated glomerular filtration rate equation based on cystatin C; eGFRcysC+cr: CKD-EPI estimated glomerular filtration rate equation based on cystatin C and serum creatinine.

Figure 1 plots each of the eGFR equations in units of mL/min/1.73m^2 according to the individual differences between mGFR and eGFRcr (A), eGFRcysC (B) and eGFRcysC+cr (C), respectively, and shows that while all measures tended to underestimate mGFR, a greater proportion of eGFRcr and eGFRcysC+cr values fell within 30% of mGFR values compared to eGFRcysC. Underestimation of mGFR by eGFRcysC occurred across the range of eGFRcysC measures (Table 2 and Figure 1).

Figure 1. Performance of eGFRcr (A), eGFRcysC (B) and eGFRcysC+cr (C) to estimate reference GFR. Reference GFR (mGFR) minus the relevant eGFR is plotted against eGFR (values above zero indicate a negative bias for eGFR).
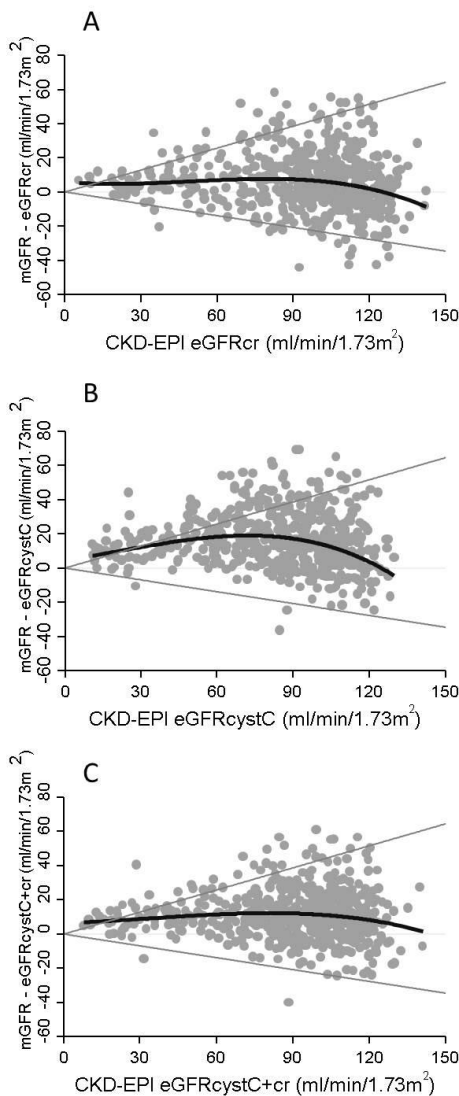
Figure note: Data inside the wedge are 'accurate' (i.e. within 30% of measured GFR). The thick lines shows the modelled (see Methods) bias.

Table 2 shows that the performance of cystatin C eGFR equations were similar for men and women, and for those aged 40-55 years and ≥55 years. Similar trends were observed for different Indigenous Australian populations (i.e. comparing Aboriginal to Torres Strait Islander Australians) (data not shown). For younger individuals aged 18-40 years, cystatin based eGFR equations did not provide any benefit in terms of bias, but eGFRcysC+cr (97.7: 94.7-99.2, p=0.02) provided significantly greater accuracy compared to eGFRcr (93.0; 88.8-96.0). There was no improvement in performance of cystatin C based eGFR equations among those with diabetes (Table 2).

When we examined the performance of eGFR equations in estimating reference GFR according to groups of adiposity, eGFRcysC and eGFRcysC+cr did not provide any benefits in terms of bias, precision or accuracy when compared to eGFRcr for individuals with a BMI ≥23kg/m^2 (Table 2) or waist circumference ≥80cm for women or ≥90cm for men (data not shown). Whilst we did observe some improvements in estimation of mGFR for eGFR equations based on cystatin C compared to eGFRcr for those with BMI <23 kg/m^2, findings were not consistent for all performance measures (Table 2). Furthermore, we did not observe the same improvements in estimation of mGFR for cystatin C equations compared to eGFRcr when adiposity was estimated using %FFM (data not shown).

When the cohort was stratified by c-reactive protein (<3, 3-10 and >10 mg/L) we found that whilst there was no significant differences in bias, % bias or accuracy for eGFRcr across increasing c-reactive protein groups, bias and % bias significantly increased with increasing c-reactive protein for eGFRcysC and eGFRcysC+cr. Accuracy for estimating mGFR also significantly deteriorated for eGFRcysC with increasing c-reactive protein (Table 3).

Table 3. Comparison of performance characteristics of eGFRcr, eGFRcysC and eGFRcr+cysC to reference GFR according to increasing c-reactive protein: the eGFR study

Estimating equation		≤3 mg/L	3-10 mg/L	>10 mg/L	P-value
n		164	219	139	
eGFRcr (mL/min/1.73m^2)	Bias	4.8 (1.7-8.7)	7.0 (2.6-9.1)	5.2 (2.1-7.9)	0.78
	% bias	6.1 (1.5-9.9)	6.6 (2.9-9.5)	5.9 (2.8-8.0)	0.89
	Precision	19.7 (-4.5 to 15.1)	22.9 (-5.0 to 17.9)	19.7 (-5.1 to 14.6)	0.88
	Accuracy	90.2 (84.6-94.3)	89.0 (84.1-92.9)	89.9 (83.7-94.4)	0.92
	% ≤30	3	5	3	
	% >30%	7	6	7	
eGFRcysC (mL/min/1.73m^2)	Bias	13.0 (11.0-15.1)	14.9 (12.4-17.4)	20.1 (16.1-24.9)	<0.001
	% bias	13.4 (11.7-16.2)	16.3 (13.1-19.4)	22.0 (18.1-26.0)	<0.001
	Precision	17.0 (4.1-21.0)	22.1 (4.8-26.9)	22.7 (9.4-32.0)	0.14
	Accuracy	82.9 (76.3-88.3)	82.2 (74.5-87.0)	71.9 (63.74-79.2)	0.029
	% ≤30	1	0	0	
	% >30%	16	17	28	
eGFRcysC+cr (mL/min/1.73m^2)	Bias	8.6 (6.5-10.7)	9.7 (7.6-12.6)	13.3 (11.0-15.4)	0.015
	% bias	9.2 (7.1-12.2)	11.5 (8.8-13.3)	15.1 (12.5-16.6)	0.017
	Precision	16.8 (1.0-17.8)	19.8 (1.0-20.8)	17.9 (5.6-23.5)	0.77
	Accuracy	93.9 (89.1-97.0)	91.3 (86.8-94.7)	89.9 (83.6-94.4)	0.43
	% ≤30	1	0	0	
	% >30%	5	8	10	

Bias and % Bias are median (95% CI); Precision is interquartile range of the bias (25th, 75th percentile of the bias); Accuracy is % of eGFR within 30% of reference GFR (95% CI).

P-values were calculated using the Mann–Whitney U-test for bias and% bias, interquantile range regression for precision, χ2-test for accuracy.

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFRcr: CKD-EPI estimated glomerular filtration rate equation based on serum creatinine; eGFRcysC: CKD-EPI estimated glomerular filtration rate equation based on cystatin C; eGFRcysC+cr: CKD-EPI estimated glomerular filtration rate equation based on cystatin C and serum creatinine

4.0 Discussion

This is the first study to assess the performance of cystatin C based eGFR CKD-EPI equations in Indigenous Australians, and provides important information on the application of eGFR equations in the detection and management of CKD in a population at high risk of ESKD. Our study of Indigenous Australians who were recruited from more than 20 sites in

urban, rural and remote regions of Australia shows that CKD-EPI eGFR equations that include cystatin C, either in isolation or in combination with serum creatinine, do not provide any overall benefits beyond CKD-EPI eGFR based on serum creatinine in estimating mGFR. These findings were observed across a range of demographic and clinical characteristics. Although there was some indication that the combined cystatin C-creatinine eGFR equation performed better than eGFRcr among those aged younger than 40 years and those with a leaner body composition, results were not consistent for all performance measures.

In our total study population, estimating equations based on cystatin C compared to those based on creatinine demonstrated significantly greater absolute and percentage bias, and no improvements in precision or accuracy were observed. These findings are in contrast to those recently reported from a large meta-analysis of predominantly Europid populations. The CKD-EPI consortium meta-analysis showed little difference between estimating equations based on creatinine, cystatin C or both in terms of bias, but significant improvements in both precision and accuracy were reported for equations based on cystatin C compared to equations based on creatinine alone [5]. However, the CKD-EPI equation based on cystatin C may not perform as well in other populations [22], and differences in population characteristics of our study population to those included in the CKD-EPI consortium analysis may explain these disparate results.

Our study population was characterised by relatively high levels of obesity, diabetes and smoking, conditions associated with inflammatory responses [23, 24]. Physiological processes unrelated to glomerular function affect both serum creatinine and cystatin C. Higher creatinine values have been associated with greater muscle mass and diets high in meat, and whilst cystatin C is less affected by these factors, other conditions associated with inflammation, including diabetes [25], smoking [26] and use of immunosuppressants [27], may affect cystatin C concentrations. C-reactive protein, a marker of inflammation, has been associated with higher values of cystatin C [25]. Obesity was highly prevalent in our study population, and few participants were lean. We found that the performance of eGFR based on cystatin C in estimating mGFR deteriorated with greater BMI and larger waist circumference. In addition, there were no significant differences for bias, accuracy or precision for eGFRcr in estimating mGFR across levels of c-reactive protein, performance estimates for cystatin C deteriorated with increasing c-reactive protein. It is therefore possible that the determinants of

cystatin C levels in this Indigenous Australian population were influenced by factors associated with chronic inflammation.

Our findings of cystatin C based equations being influenced by obesity are supported by other studies [28] including Asian and Indigenous populations [29-31]. One observational study of immigrant South Asians was also able to demonstrate that the relationship between increasing adiposity and eGFRcysC was largely explained by factors related with chronic inflammation, including c-reactive protein [30]. In another study of Indigenous youth, cystatin C was strongly associated with the metabolic syndrome [31]. In contrast to our findings, however, other studies of Asian populations have reported that the combined eGFRcysC+cr equation was superior to eGFRcr [11, 32, 33]. The authors from the study of a multiethnic Asian population postulated that in that population of individuals from diverse Asian heritages, the estimation of mGFR was improved because markers of both fat and muscle, as represented by cystatin C and creatinine, respectively, were included [11]. Therefore, the relative impact of these opposing physiologies may need to be taken into account when assessing the appropriateness of GFR estimating equations in different ethnic populations.

The eGFR study is the largest study to investigate the performance of cystatin C based eGFR in estimating mGFR in Indigenous Australians. These findings may be generalisable to Indigenous Australians living in urban, rural and remote locations, as well as those with and without diabetes and kidney disease. Nevertheless, limitations exist. Firstly, the representativeness of the study population is unknown, as participants were volunteers and not randomly selected. Secondly, serum cystatin C can also be affected by thyroid function and corticosteroids, but we were not able to ascertain the influence of these factors. Thirdly, the renal measures and mGFR were only taken once, and given that measurement error is reported to be 5-20%, excess variability in measurements may have attenuated our study findings.

The results of our study indicate that compared to CKD-EPI eGFR based on serum creatinine, cystatin C based eGFR equations demonstrated poorer performance in estimating mGFR. Nevertheless, eGFR equations based on cystatin C may demonstrate a different relationship with renal and cardiovascular disease, and overall mortality outcomes. A recent meta-analysis revealed that compared to eGFRcr, cystatin C based eGFR equations were more strongly associated with cardiovascular disease, all-cause mortality and ESKD [34]. These findings that are based predominantly on North American and European populations

have also been observed in other ethnicities that are at high risk for kidney disease including older Mexican Americans [35] and Pima Indians with type 2 diabetes from the United States [36]. Given the high prevalence of conditions and behaviours associated with elevated chronic inflammation observed in our population of Indigenous Australians, further analysis of the role of cystatin C in predicting renal and mortality outcomes should also be assessed.

5.0 Conclusions

In conclusion, accurate estimation of renal function in Indigenous Australians is vital to identifying patients for clinical management. Our findings indicate that the addition of serum cystatin C either in isolation or together with serum creatinine does not improve the performance of eGFR equations in estimating GFR in Indigenous Australians. Therefore, we support the continued use of CKD-EPI eGFR equations based on serum creatinine in Aboriginal and Torres Strait Islander Australians and do not support the use of cystatin C based equations for further analysis of specific subgroups.

6.0 Abbreviations

BMI	Body mass index
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
eGFR	Estimated glomerular filtration rate
eGFRcr	Estimated glomerular filtration rate calculated using the CKD-EPI equation for serum creatinine
eGFRcysC+cr	Estimated glomerular filtration rate calculated using the CKD-EPI equation for cystatin C and serum creatinine
eGFRcystC	Estimated glomerular filtration rate calculated using the CKD-EPI equation for cystatin C
ESKD	End-stage kidney disease
FFM	Fat free mass
GFR	Glomerular filtration rate
HbA$_{1c}$	Haemoglobin A$_{1c}$
KDIGO	Kidney Disease Improving Global Outcomes
mGFR	Reference glomerular filtration rate determined using non-isotopic iohexol plasma disappearance technique over 4 hours

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