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Cystatin C- and creatinine-based equations in the assessment of renal function in HIV positive patients prior to commencing Highly Active Antiretroviral Therapy (HAART)

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Guarantor: JAG

Contributorship: T S collected the data, obtained ethical permission and wrote the first draft. G C carried out the radioactive studies and contributed to the final draft, V G and H E van Deventer carried out statistical analysis, interpretation and revisions to the article and J A George conceptualized the study, interpreted data and revised the article. All authors reviewed and edited the manuscript and approved the final version of the manuscript

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Abstract:

Background: We evaluated the accuracy and precision of creatinine- and cystatin C-based prediction equations for estimating glomerular filtration rate (eGFR) compared to measured GFR (mGFR) in an antiretroviral-naive human immunodeficiency virus (HIV) population.

Methods: The study population consisted of 100 treatment-naive HIV patients. GFR was estimated using the Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations as well as cystatin C-based equations (CKD-EPI $_{\text{cystatin C}}$, cystatin C $_{\text{van Deventer}}$ and CKD-EPI $_{\text{combined}}$) compared to ^{51}Cr-EDTA plasma clearance mGFR. We calculated percentage bias, standard deviation (SD) of the differences, accuracy within 15% and 30% of mGFR and sensitivity and specificity for predicting mGFR < 60 mL/min/1.73m^2.

Results: Bias for all eGFR equations ranged from -9.4 % to 38.4 %. The CKD-EPI $_{\text{combined}}$ without ethnicity correction factor equation had the least bias, 2.9% (-2.9 to 8.8). Bias was higher for the MDRD and CKD-EPI equation with the African American ethnicity factor (38.4% and 33.7%) than without (14.2% and 15.3%). SD of the differences ranged from 29.2 % (CKD-EPI $_{\text{combined}}$ without ethnicity factor) to 54.0% (MDRD with ethnicity factor). Accuracy within 30% of mGFR ranged from 78% for CKD-EPI $_{\text{combined}}$ without ethnicity factor to 56.7% for the Cockcroft-Gault equation. Sensitivity for creatinine based equations was less than 50% and for the CKD-EPI $_{\text{cystatin C}}$ equation was 75%.

Conclusion: Sensitivity of creatinine-based equations for predicting GFR was poor in this group of patients. The CKD-EPI $_{\text{combined}}$ equation performed better than creatinine-based equations.

Keywords: estimated GFR, cystatin C, HIV, MDRD, prediction equations

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INTRODUCTION

Human immunodeficiency virus (HIV) infection affects almost 25 million people in Sub-Saharan Africa (1). While the introduction of antiretroviral therapy has resulted in a significant reduction of acquired immune deficiency syndrome (AIDS) related deaths (2, 3), disease conditions such as chronic kidney disease (CKD) have emerged as important causes of morbidity and mortality in the United States and Europe (4). Renal dysfunction progressing to end-stage renal disease is a common complication of HIV infection (5, 6). In Africa, a wide spectrum of renal diseases that have been described on biopsies obtained from infected patients (7, 8). The classic kidney disease of HIV infection is HIV associated nephropathy (HIVAN) (9, 10) which results in accelerated progression to AIDS and increased mortality. Treatment of HIV and comorbidities may also lead to renal disease e.g. the use of tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor (11).

In addition to the effects of HIV infection and its therapy on the kidney, chronic diseases like diabetes and hypertension add to the risk of CKD in Africa. The impact of both infectious diseases like HIV and chronic diseases on the prevalence of CKD has huge public health implications for Africa (12). Studies suggest that reduced glomerular filtration rate (GFR) is very prevalent among HIV infected people in Africa (13, 14) but there is substantial variability between the methods used to estimate GFR (10, 13, 15, 16). In Africa renal function is most commonly assessed by either the Modification of Diet in Renal Disease (MDRD) study equation (17) or the Cockcroft-Gault equation (18). More recently the creatinine based CKD-EPI equation (CKD-EPI) was shown to estimate GFR (eGFR) more accurately than the MDRD equation (19) and has replaced the MDRD equation for estimating GFR in some laboratories, but its use has not been validated in local populations. In 2012, Inker et al, demonstrated in a large cross-sectional analysis that a combined creatinine- and cystatin C-based equation (CKD-EPI $_{combined}$) estimated GFR more accurately than the CKD-EPI equation (20).

Cystatin C is a low molecular weight (13kD) non-glycosylated basic protein produced by all nucleated cells (21). It is produced at a constant rate, is freely filtered by the glomerulus and does not re-enter the circulation after being filtered through the glomerulus (22). It therefore meets many of the key criteria of

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an ideal endogenous glomerular filtration rate marker. Cystatin C production is independent of muscle mass and dietary influences and cystatin C-based prediction equations is therefore potentially not subject to some of the limitations of serum creatinine-based eGFR equations (23). A previous study from our centre showed that cystatin C-based prediction equations are more precise than serum creatinine-based equations for patients in predicting eGFR in patients with measured GFR (mGFR) > 60 ml/min/1.73m^2 (24). Cystatin C may therefore be of benefit in detection of early renal dysfunction. As patients with HIV are at increased risk for the development of CKD, it is important that eGFR equations can accurately estimate GFR and identify patients with possible CKD.

The aim of this study was to evaluate the use of commonly described eGFR prediction equations in an antiretroviral naive HIV positive population using ^{51}Cr-EDTA plasma clearance as the reference mGFR.

Materials and Methods:

Sample collection: This cross-sectional study was conducted on samples collected from one hundred treatment naïve HIV positive adult medical inpatients at the Chris Hani Baragwanath Hospital, Soweto, South Africa. Exclusion criteria were: patients receiving HAART (currently and those who defaulted treatment), pregnant or breastfeeding individuals and patients with one or more of the following conditions hypertension, diabetes mellitus, oedema or presence of known renal complications with current admission. The Human Research Ethics Committee of the University of the Witwatersrand (Clearance certificate number M10410) approved the study. We obtained written informed consent from all patients prior to enrolment. Participants' clinical history, age, height and gender were available from medical records.

Patients were fasting from the night before. Five mL of EDTA plasma and five mL of serum was collected from each participant for cystatin C and creatinine measurement respectively between 8:00 am and 10 am. Samples were centrifuged at 3500 rpm for 10 minutes and stored at -70^{0}C until analysis.

GFR Measurement: GFR measurement was performed by the Nuclear Medicine department using ^{51}Cr-EDTA according to published guidelines (25). After injection of 3.7 MBq ^{51}Cr-EDTA intravenously blood samples were collected from the contralateral arm at 120 and 240 minutes post injection. GFR was measured with the slope intercept method. The Brochner-Mortensen equation was used according to

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guidelines adopted by the British Nuclear Medicine Society to correct the obtained measurements (26). The DuBois method BSA (m^2) = [71.84 weight (kg) $^{0.425}$ * height (cm) $^{0.725}$] / 10 000 was used to normalize GFR to the body surface area (27).

Creatinine and cystatin C measurement: Cystatin C was measured on a Siemens Advia 1800 analyser (Siemens Healthcare Diagnostics, Tarrytown, USA) by an automated latex-enhanced immune-nephelometric assay traceable to the IFCC reference method (ERM-DA471). Creatinine was measured spectrophotometrically using the kinetic modified Jaffe method on the Siemens Advia 1800 analyser (Siemens Healthcare Diagnostics, Tarrytown). The creatinine assay is traceable to an isotope dilution mass spectrometry reference creatinine method (28). All samples were analysed in duplicate.

GFR estimation: Glomerular filtration rate was estimated using the Cockcroft-Gault equation (18) normalized to 1.73m^2, MDRD equation (IDMS traceable) with (29) and without ethnicity factor (30), the CKD-EPI equation (19), the cystatin C $_{\text{van Deventer}}$ equation (24), the CKD-EPI $_{\text{cystatin C}}$ equation and the CKD-EPI $_{\text{combined}}$ equation (20). (Refer to Supplementary material for full equations used)

Statistical Analysis

The Shapiro Wilk test was used to assess normality of data. Continuous data variables are expressed as mean ±SD if parametric and median (interquartile range, IQR) if non-parametric. Difference plots were used for comparison studies. For the difference plots mean percentage bias, SD of the difference and 95% limits of agreement were calculated for each of the equations. Wilcoxon match pairs signed ranks test was used to test for significance of bias compared to reference mGFR. Accuracy within 15% (P_{15}) and within 30% (P_{30}) was also calculated for each of the equations. The McNemar test was used to compare P_{15} and P_{30} values. Receiver operating characteristic (ROC) curve analysis was used to calculate the sensitivity and specificity to correctly predict mGFR < 60 mL/min/1.73m^2. Statistical analysis was performed using the MedCalc Statistical program (MedCalc Version 11.6.1, Mariakerke, Belgium) and Analyse-It (Analyse-It Software Ltd Version 2.26, Leeds UK).

Results

One hundred black South African HIV positive patients were enrolled in the study. Results of ninety-seven patients are presented. Three patient results were excluded due to incomplete data. All participants were inpatients admitted at the Chris Hani Baragwanath hospital for a wide range of diseases except for

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primary renal disease. The majority (43/97, 44%) of the patients were admitted with lower respiratory tract infection, followed by tuberculosis in 25% (24/97) and opportunistic infections such as cryptococcal meningitis in 14% (14/97). Minority of patients had diagnoses that included lymphomas and bacterial and viral meningitis.

Basic characteristics of the group are presented in Table 1. mGFR ranged between 24.7 and 164.6 and mL/min/1.73m^2. Of note 16% (n=16) of patients had an mGFR of less than 60 ml/min/1.73m^2 and 50% of the group had a mGFR greater than 90 ml/min/1.73m^2.

Comparisons of the different eGFR equations:

Bias and difference plots

Most of the creatinine based equations evaluated overestimated mGFR in this population. Inclusion of the ethnicity factor resulted in a greater overestimation of mGFR as compared to when the ethnicity factor was excluded (Table 2 and Figure 1). The MDRD equation overestimation was 38.4 (27.5 to 49.3) % when the ethnicity factor was included vs. 14.2 (5.2 to 23.2) % without ethnicity factor. The CKD-EPI equation overestimation was 33.7 (25.0 to 42.4) % with the ethnicity factor vs. 15.3 (7.8 to 22.8) % without. For the CKD-EPI $_{combined}$ equation overestimation was 11.5 (5.4 to 17.6) % with the ethnicity factor vs. 2.9 (-2.9 to 8.8) % without the ethnicity factor. Using the Wilcoxon match pairs signed ranks test, only the CKD-EPI $_{combined}$ without ethnicity factor equation had no significant bias when compared to mGFR. The cystatin C based equations (CKD-EPI $_{cystatin\ C}$ and cystatin C $_{van\ Deventer}$) underestimated GFR in this population, -5.5 (-11.7 to 0.7) % and -9.4 (-15.5 to -3.2) % respectively.

SD of the differences appeared to be higher for creatinine based equations compared to cystatin C based equations (mean SD of creatinine based equations was 39.1% compared to 29.9% for cystatin C based equations). The cystatin C-based equations (CKD-EPI $_{combined}$, CKD-EPI $_{cystatin\ C}$ and cystatin C $_{van\ Deventer}$) showed a concentration bias effect, i.e. more positively biased at lower mGFR. There was no concentration bias effect for the MDRD or Cockcroft-Gault equation.

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Accuracy within 15% and 30% of mGFR

Accuracy within 30% of mGFR ranged from 56.7% for the Cockcroft-Gault equation to 78.0% for the CKD-EPI $_{combined}$ without ethnicity factor equation (Table 2). Using the McNemar test accuracy within 30% was higher for the MDRD equation without ethnicity factor (59.8%) than for the MDRD equation with ethnicity factor, 48.3% (P=0.001). P_{15} and P_{30} was significantly better for the CKD-EPI equation without the ethnicity factor (35.1% and 62.9%) compared to the CKD-EPI equation with ethnicity factor, 24.7% and 41.2% (P=0.05 and P<0.0001 respectively). Using the McNemar test P_{15} and P_{30} was not significantly different between the MDRD equation without ethnicity factor and the CKD-EPI equation without ethnicity factor. P_{15} for the CKD-EPI $_{cystatin\ C}$ equation (42.3%) was better than for the MDRD equation without ethnicity factor, 27.8% (P=0.007) but not statistically better than the CKD-EPI equation without ethnicity factor (35.1%). P_{15} and P_{30} were significantly better for the CKD-EPI $_{combined}$ without ethnicity factor equation than for the CKD-EPI equation without ethnicity factor (P=0.003 and P=0.002 respectively) (Table 2 and Addendum Table 1).

Sensitivity for predicting mGFR < 60 mL/min/1.73m^2

While none of the creatinine bases equations had a good sensitivity for predicting mGFR < 60 mL/min/1.73m^2 (Table 2), sensitivity was higher for cystatin C based equations. Sensitivity for the CKD-EPI $_{cystatin\ C}$ equation was 75% and for the CKD-EPI $_{combined}$ equation without ethnicity factor it was 69%. For all creatinine based equations sensitivity was less than 50%.

Discussion

Here we report on the performance of GFR estimating equations using creatinine and cystatin C with and without the ethnicity factor in a group of anti-retroviral naive HIV positive patients. The results of this study show that eGFR varies depending on the equation used and that cystatin C based equations perform better than creatinine based equations. This is in keeping with a number of other studies that have shown cystatin C to be superior to creatinine only based equations in the general population (24,

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31) as well as in HIV infected individuals (32, 33). These results have implications for the clinical use of GFR estimating equations in the HIV positive population.

In the context of HIV infection, impaired kidney function develops as a result of various risk factors including aging, genetic factors and the burden of HIV infection itself. The World Health Organization recommends screening for renal disease using eGFR prior to initiation of therapy with TDF (34). The most commonly used equations are the MDRD and the Cockcroft-Gault equations, which result in overestimation of mGFR. Following a review of the current literature, the authors could not find recommendations as to which eGFR equation should be used for patients initiating TDF therapy although all guidelines recommend that GFR should be estimated at the beginning of therapy and periodically thereafter.

The MDRD equations has been shown to have varying accuracy in different population groups (30, 35, 36).This has been attributed to variations in non-GFR determinants of serum creatinine such as muscle mass and diet which may be affected by acute and chronic disease (37). In our study the CKD-EPI equation was not superior to the MDRD equation in antiretroviral naive HIV positive patients. This is in contrast to Inker et al. who noted that the CKD-EPI equation performed better than the MDRD equation in HIV infected patients on antiretroviral therapy (20). They used iohexol clearance as a gold standard on clinic patients while we used ^{51}Cr-EDTA plasma clearance and our patients were in patients. A systematic review of estimating equations for GFR showed that neither the MDRD nor the CKD-EPI is optimal for all population groups but that the use of the CKD-EPI would lead to a smaller average bias in clinical practice (38). It remains to be determined if this is true in African populations.

In this study, including the African American ethnicity factor in the MDRD and CKD-EPI equations the resulted in an overestimation of mGFR which improved when the ethnicity factor was not included. An explanation for this may be the patient population selected, who are HIV infected hospital in-patients and thus more likely to have decreased muscle mass due to muscle wasting and malnutrition (39, 40). The MDRD ethnicity factor was based on an African American population (largely descendant from West Africa) with CKD and hence is applicability is likely not universal to all African populations and those without CKD.

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Wyatt et al have previously investigated estimation of GFR in an ARV naïve population, using iohexol clearance for measured GFR (36). Accuracy within 30% of mGFR was 83% when the ethnicity factor was not used compared to 73% with the race coefficient (36). This is higher than the accuracy shown in the present study. It is not clear if the alkaline picrate method they used was IDMS traceable. They used dried blood spot measurement of iohexol clearance as a gold standard on clinic patients while we used ^{51}Cr-EDTA plasma clearance. Madala et al. have also shown, using (99m)Tc-DTPA-measured GFR, that inclusion of the African-American ethnicity correction factor in black South Africans resulted in 17.1% overestimation of mGFR compared to 5% without the use of the ethnicity factor (41).

Cystatin C concentration is considered independent of muscle mass and dietary influences which may explain the better performance in our population subgroup. Of note, the mean BMI of the group was 20.9 kg/m^2. While cystatin C is independent of muscle mass, it may be influenced by adiposity and by inflammation (42). This may explain the different performance of cystatin C based equations observed in a number of studies. In transplant patients, cystatin C based equations gave better 30% and 50% accuracy compared with creatinine based equations (43). Only a few studies have looked at the performance of cystatin C based equations in HIV patients often with contradictory results. In HIV patients on treatment, Inker et al showed that cystatin C was less accurate for GFR < 60 ml/min/1.73m^2 (44), while in a small group of HIV positive patients the cystatin C $_{\text{van Deventer}}$ equation was more precise than the MDRD or the CKD-EPI equation (24). Similarly, cystatin C eGFR was more precise than MDRD in a group of Thai HIV patients (45). Driver et al showed that cystatin C eGFR were more strongly associated with mortality risk than creatinine eGFR (33).

There are limitations to our study, one being the relatively small sample size. As a result, we had very few patients within each CKD group and most of the study cohort did not have CKD. However although most patients in the study did not have GFR < 60 ml/min/1.73m^2, a study by Jose et al. examining the decline of renal function following initiation of TDF demonstrated that an eGFR of < 75 mL/minute/1.73 m^2 at the start of therapy was associated with an increased risk of discontinuing TDF and an eGFR of < 90 mL/min/1.73 m^2 at the time of discontinuation was associated with an increased risk of incomplete reversibility (46). Jose et al advise that renal monitoring during TDF therapy and discontinuation for

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those with declining renal function (46), hence the importance of using as accurate as possible means of estimating GFR in this group. We did not measure urinary protein excretion. Furthermore, the study was conducted in inpatients although GFR measurements were not carried out when they were acutely ill. Strengths of the study are that GFR was measured using a ^{51}Cr-EDTA plasma clearance method and that the study was able to evaluate various commonly used eGFR equations (both creatinine based and cystatin C based) in a population (African HIV positive antiretroviral naïve patients) for whom accurate eGFR measurement is important.

Epidemiological studies have shown that the prevalence of stage 3 CKD is about 10% in the western world (47, 48). Whilst population based data on the burden of CKD in Africa is lacking, a systematic review and meta-analysis of 21medium and high quality studies from Africa noted a prevalence of 13.9% (95%CI 12.1-15.7) (49). In this study, using mGFR, 16% of all patients had GFR < 60 mL/min/1.73 m^2. The estimated prevalence varies depending on the method used to estimate GFR (50). Among the elderly and in the general population the prevalence was shown to be much higher when the MDRD formula was used compared to when cystatin C based equations were used (51, 52). We also showed that that eGFR in our HIV infected population differed based on the eGFR equation used. Moving from the MDRD equation to the CKD-EPI $_{combined}$ will decrease the estimated prevalence of CKD, which has major public health implications (53). In our study, cystatin C based prediction equations had a smaller bias compared to creatinine based equations with the smallest bias was observed for the CKD-EPI $_{combined}$ equation. Given that cost is a prohibitive factor in Africa it may not be practical at this stage to recommend the widespread use of cystatin C and we were unable to identify a subgroup that may benefit from it. In the South African context the use of CKD-EPI or MDRD equations without ethnicity factor may be the most practical option for estimating GFR.

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Legend: Figure 1

Difference plots: **A:** MDRD with ethnicity factor **B:** MDRD without ethnicity factor. **C:** CKD-EPI with ethnicity factor. **D:** CKD-EPI without ethnicity factor. **E:** CKD-EPI $_{\text{cystatin C}}$ **F:** van Deventer $_{\text{cystatin C}}$ **G:** CKD-EPI $_{\text{combined}}$ with ethnicity factor **H:** CKD-EPI $_{\text{combined}}$ without ethnicity factor

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Table 1: Participant Characteristics

	Male (N=57) ***Mean ± SD or*** ***Median (IQR)***	***Female (N=40)*** ***Mean ± SD or*** ***Median (IQR)***	***Combined (N=97)*** ***Mean ± SD or*** ***Median (IQR)***
Age, years	39.6 ± 9.2	33.2 ± 9.0	37.0 ± 9.6
Weight, kg	58.7 (12.4)	56.8 (25.5)	57.5 (14.4)
Height, cm	169 (9.9)	157 (6.8)	165.0 (12.3)
Body surface area, m^2	1.69 ± 0.15	1.62 ± 0.18	1.66 ± 0.16
Body mass index (BMI), kg/m^2	20.2 (3.5)	20.1 (9.7)	20.9 (5.1)
S-creatinine, µmol/L	87.0 (38.0)	60.8 (21.1)	72.5 (43.3)
S-cystatin C, mg/L	1.0 (0.4)	1.0 (0.4)	1.0 (0.4)
CD4, / uL	152 (256)	185 (282)	162 (280)
mGFR, $mL/min/1.73m^2$	88.1 (37.0)	95.4 (38.4)	92.5 (38.3)
Classification: mGFR	N (%)	N (%)	***N (%)***
mGFR < 60 $ml/min/1.73m^2$	9 (16%)	7 (18%)	16 (16%)
mGFR 60-90 $ml/min/1.73m^2$	21 (37%)	10 (25%)	31 (32%)
mGFR > 90 $ml/min/1.73m^2$	27 (47%)	23 (58%)	50 (52%)
Classification: CD4	N (%)	N (%)	***N (%)***
≥ 500 / uL	32 (57%)	20 (53%)	52 (55%)
200 – 499 / uL	18 (32%)	17 (45%)	35 (37%)
< 200 / uL	6 (11%)	1 (3%)	7 (7.4%)

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Table 2: Performance of the eGFR equations compared to mGFR

	Bias (%) (95% CI)	*Median difference, mL/min/1.73m2 (95% CI)*	*P*[1]	*SD of difference*	*95% Limit of Agreement*	P_{15}	P_{30}	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Cockcroft-Gault	22.1 (12.7 to 31.5)	14.5 (7.8 to 21.4)	< 0.0001	46.7%	-69.3% to 113.6%	34.0	56.7	0.38 (0.15 to 0.65)	0.96 (0.90 to 0.99)
MDRD – with ethnicity factor	38.4 (27.5 to 49.3)	28.2 (20.5 to 36.7)	< 0.0001	54.0%	-67.4% to 144.3%	22.7	43.3	0.31 (0.11 to 0.59)	0.99 (0.93 to 1.00)
MDRD – without ethnicity factor	14.2 (5.2 to 23.2)	15.0 (3.5 to 25.9)	0.01	44.6%	-73.1% to 101.6%	27.8	59.8	0.44 (0.20 to 0.70)	0.95 (0.88 to 0.99)
CKD-EPI – with ethnicity factor	33.7 (25.0 to 42.4)	26.7 (20.8 to 32.0)	< 0.0001	43.2%	-51.1% to 118.4%	24.7	41.2	0.31 (0.11 to 0.59)	0.99 (0.93to 1.00)
CKD-EPI – without ethnicity factor	15.3 (7.8 to 22.8)	10.2 (5.2 to 15.4)	< 0.0001	37.3%	-57.8% to 88.4%	35.1	62.9	0.31 (0.11 to 0.59)	0.98 (0.91 to 1.00)
***CKD-EPI** $_{cystatin\ C}$*	-5.5 (-11.7 to 0.7)	-6.6 (-11.2 to -2.1)	0.005	30.9%	-66.1% to 55.1%	42.3	75.3	0.75 (0.48 to 0.93)	0.90 (0.82 to 0.96)
***Cystatin C** $_{van\ Deventer}$*	-9.4 (-15.5 to -3.2)	-11.3 (-15.2 to -7.3)	< 0.0001	30.4%	-68.9% to 50.2%	45.4	72.2	0.63 (0.35 to 0.85)	0.93 (0.85 to 0.97)
CKD-EPI** $_{combined}$ **– with ethnicity factor	11.5 (5.4 to 17.6)	8.4 (4.4 to 12.7)	< 0.0001	30.3%	-47.8% to 70.9%	44.0	73.0	0.56 (0.30 to 0.80)	0.96 (0.90 to 0.99)
CKD-EPI** $_{combined}$ **– without ethnicity factor	2.9% (-2.9 to 8.8)	-0.7 (-4.5 to 3.4)	0.52	29.2%	-54.3% to 60.2%	51.0	78.0	0.69 (0.41 to 0.89)	0.95 (0.88 to 0.99)

[1] Wilcoxon match pairs signed ranks test compared to mGFR

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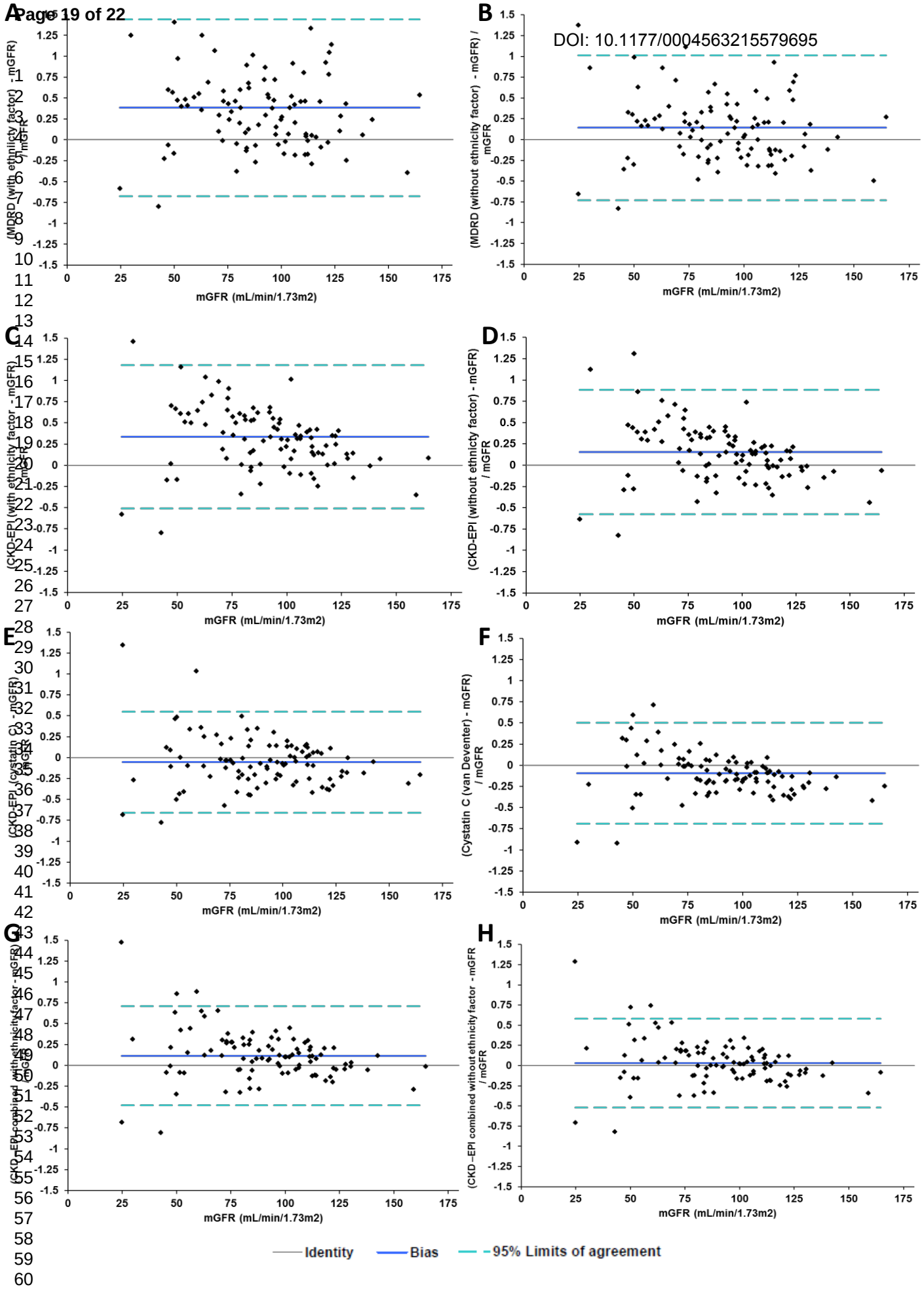

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Supplementary material

a) Cockcroft-Gault equation:

eGFR = (140 - age) x body weight (kg)/a x serum creatinine in μmol/L x 1.73 m^2/BSA

where: a = 0.8 for men and 0.85 for women and BSA is body surface area

b) MDRD equation:

eGFR = 175 x (serum creatinine in μmol/L/88.4)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x 1.212 if African-American

c) CKD-EPI equation (2009):

eGFR = 141 x min (S_{cr} /κ, 1)$^{\alpha}$ x max(S_{cr} /κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if black]

where: S_{cr} is serum creatinine in μmol/L, κ is 61.9 for females and 79.6 for males, α is -0.329 for females and -0.411 for males,

d) CKD-EPI $_{cystatin\ C}$ equation (2012):

eGFR = 133 x min(SCysC/0.8, 1)$^{0.499}$ max(SCysC/0.8, 1)$^{1.328}$ x 0.996^{Age} [x 0.932 if female]

where: SCysC is serum cystatin C (in mg/L)

e) Cystatin C $_{van\ Deventer}$ eGFR equation:

eGFR = $10^{2.35}$ x $10^{(SCysC\ x-0.33)}$ * $10^{(-0.003\ x\ Age)}$

where: SCysC is serum cystatin C (in mg/L)

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f) CKD–EPI $_{combined}$ equation (2012):

$eGFR = 135 \times \min(SCr/k, 1)^{a} \times \max(SCr/k, 1)^{-0.601} \times \min(SCysC/0.8, 1)^{-0.375} \times \max(SCysC/0.8, 1)^{-0.711} \times 0.995^{Age}$ [x 0.969 if female] [x 1.08 if black]

where: SCr is serum creatinine (in mg/dL), SCysC is serum cystatin C (in mg/L), k is 0.7 for females and 0.9 for males, a is 0.248 for females and 0.207 for males

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Addendum: Table 1: P value for McNemar test; Comparing P_{30} and P_{15} respectively

	Cockcroft-Gault	*MDRD – with ethnicity factor*	*MDRD – without ethnicity factor*	*CKD-EPI – with ethnicity factor*	*CKD-EPI – without ethnicity factor*	*CKD-EPI cystatin C*	*Cystatin C van Deventer*	CKD-EPI combined – with ethnicity factor	CKD-EPI combined – without ethnicity factor
Cockcroft-Gault		0.0009 and 0.08	0.65 and 0.26	0.003 and 0.12	0.29 and 1.00	0.02 and 0.14	0.004 and 0.31	0.001 and 0.09	< 0.0001 and 0.005
MDRD – with ethnicity factor			0.001 and 0.61	0.58 and 0.21	< 0.0001 and 0.04	< 0.0001 and 0.005	< 0.0001 and 0.01	< 0.0001 and 0.002	< 0.0001 and < 0.0001
MDRD – without ethnicity factor				0.005 and 0.74	0.69 and 0.21	0.08 and 0.008	0.02 and 0.03	0.005 and 0.74	0.0008 and <0.0001
CKD-EPI – with ethnicity factor					< 0.0001 and 0.05	< 0.0001 and 0.007	< 0.0001 and 0.02	< 0.0001 and 0.0008	< 0.0001 and < 0.0001
CKD-EPI – without ethnicity factor						0.18 and 0.14	0.06 and 0.37	0.02 and 0.10	0.002 and 0.003
CKD-EPI cystatin C							0.45 and 0.63	0.69 and 1.00	0.10 and 0.28
Cystatin C van Deventer								1.00 and 0.79	0.36 and 0.15
CKD-EPI combined – with ethnicity factor									0.12 and 0.19
CKD-EPI combined – without ethnicity factor									