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Estimating Glomerular Filtration Rate in Kidney Transplant Recipients: Comparing a Novel Equation With Commonly Used Equations in this Population

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Background. Assessment of glomerular filtration rate (GFR) is important in kidney transplantation. The aim was to develop a kidney transplant specific equation for estimating GFR and evaluate against published equations commonly used for GFR estimation in these patients. **Methods.** Adult kidney recipients (n = 594) were included, and blood samples were collected 10 weeks posttransplant. GFR was measured by ^{51}Cr-ethylenediaminetetraacetic acid clearance. Patients were randomized into a reference group (n = 297) to generate a new equation and a test group (n = 297) for comparing it with 7 alternative equations. **Results.** Two thirds of the test group were males. The median (2.5-97.5 percentile) age was 52 (23-75) years, cystatin C, 1.63 (1.00-3.04) mg/L; creatinine, 117 (63-220) μmol/L; and measured GFR, 51 (29-78) mL/min per 1.73 m^2. We also performed external evaluation in 133 recipients without the use of trimethoprim, using iohexol clearance for measured GFR. The Modification of Diet in Renal Disease equation was the most accurate of the creatinine-equations. The new equation, estimated GFR (eGFR) = 991.15 × (1.120^{sex}/([age$^{0.097}$] × [cystatin C$^{0.306}$] × [creatinine$^{0.527}$]); where sex is denoted: 0, female; 1, male, demonstrating a better accuracy with a low bias as well as good precision compared with reference equations. Trimethoprim did not influence the performance of the new equation. **Conclusions.** The new equation demonstrated superior accuracy, precision, and low bias. The Modification of Diet in Renal Disease equation was the most accurate of the creatinine-based equations.

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Assessment of glomerular filtration rate (GFR) is important in the follow-up of patients after receiving a kidney transplant. Many equations based on plasma markers are in use for estimating GFR in different patient groups, but there is still a need for a specific and accurate equation for use in kidney transplant recipients.[1,2] Several equations are based on the endogenous substance creatinine.[3-6] It is well known that the plasma level of creatinine is affected by muscle mass[7,8] and ingestion of protein or creatine,[9,10] in addition to the GFR. Plasma creatinine is also somewhat limited as a marker for GFR since it is subjected to a certain degree of tubular secretion.[11] Trimethoprim is known to inhibit tubular creatinine secretion leading to rapid and reversible increase in serum creatinine and falsely underestimation of GFR, at least in doses above 160 mg.[12] The endogenous protein cystatin C has also been used as a marker for renal function with the advantage that it is less dependent on muscular mass.[13,14] Thus, cystatin C can be used as an alternative, or incorporated as an auxiliary marker, for estimating GFR in patients with low muscle mass (children, elderly, patients with anorexia,

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amputations, or paresis) or high muscle mass (bodybuilders). Cystatin C is also less influenced by renal tubular secretion and may be a good alternative to creatinine-based equations in situations where tubular excretion of creatinine is affected (eg, drugs blocking the tubular creatinine transporter such as trimethoprim).[12,15] However, different studies have reported that cystatin C could be influenced by factors independent of GFR, such as the level of corticosteroids, thyroid hormones,[16-19] sex, diabetes, and inflammation.[13] On the other hand, one study claims that the inflammatory status of a patient does not influence cystatin C levels.[20] If a more accurate determination of GFR is needed, an exogene marker should be used, such as clearance of the nonradioactive substances, like the contrast agent iohexol or inulin or radiolabeled agents, like ^{51}Cr-ethylenediaminetetraacetic acid (^{51}CrEDTA), ^{99m}Tc-diethylenetriamine-pentaacetate (^{99m}TcDTPA) or ^{125}I-iothalamate, but these techniques are invasive, time consuming and costly.[21]

Several studies have evaluated the performance of different well-known GFR estimating equations (eGFR-equations), both based on creatinine and cystatin C or their combination, in kidney transplant recipients.[1,2,22-27] The most commonly used equations in kidney transplant recipients are the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)$_{creatinine}$ and Cockcroft-Gault equations.[3-5] The MDRD equation was derived from 1628 patients with chronic kidney disease. It is well known that the formula underestimates GFR in patients with high GFR values.[28,29] The CKD-EPI $_{creatinine}$ equation was derived from participants with higher GFR, a mean GFR of 68 mL/min per 1.73 m^2. Only a minor fraction (4%) of the cohort was kidney transplant recipients. The Cockcroft-Gault formula was generated from 236 patients, mainly males, but the method for measurement of creatinine was "Jaffe" which is subject to errors from interfering compounds. The Nankivell formula is the only equation derived from kidney transplant recipients, but only in 146 recipients and with repeated measurements. This equation does not include Cystatin C and is not widely used in follow-up of renal transplant recipients around the world.[6]

The purpose of our study was therefore to generate a new equation for use in adult kidney recipients and to evaluate the performance of different eGFR-equations, based on creatinine, cystatin C and a combination of both, with the measured GFR (mGFR) by ^{51}CrEDTA clearance. We also performed external evaluation in a different cohort of kidney recipients, not using trimethoprim, from a more recent period with iohexol-based GFR measurements as the standard.

MATERIALS AND METHODS

Study Population

A total of 594 adult kidney recipients were included in the study between 2005 and 2009 at Oslo University Hospital. The examinations were done 10 weeks after transplantation in a stable clinical situation. At that time the immunosuppression consisted of either cyclosporine A or tacrolimus in combination with mycophenolate and steroids. Cyclosporine was C2 monitored with a therapeutic window of 1000 to 1200 μg/L and tacrolimus was C0 monitored with a therapeutic window of 5 to 10 μg/L. In combination with cyclosporine, 1.0 g mycophenolate mofetil was given twice daily and in combination with tacrolimus, the dose was 0.75 g twice daily. All patients received prophylactic trimethoprim-sulfamethoxazole (80 mg trimethoprim per day) for a total of 6 months from the first day after transplantation. The study population was randomly divided into 2 groups; a reference group of 297 patients to generate a new equation, and a test group of 297 patients entirely used to compare the different estimating equations including evaluation of our new equation. Median prednisolone dose was 10 mg, both in the reference group and the test group. Cyclosporine was given to 57% of the patients, both in the reference group and the test group. Additionally, an external evaluation group consisting of 133 kidney recipients transplanted between 2014 and 2016 at Oslo University Hospital was included. The examinations in this cohort were performed 8 weeks and 1 year posttransplant. Measured GFR was determined by iohexol clearance in these patients and they received concomitant trimethoprim at the 8-week investigation, but not at the 1-year investigation. Immunosuppression consisted of steroids (median daily dose, 10 mg at 8 weeks and 5 mg at 1 year), tacrolimus (median concentration, 6.0 μg/L at 8 weeks and 6.2 μg/L at 1 year) and a median dose of 750 mg mycophenolate mofetil at both investigations.

Approval of the study was obtained from the Regional Committees for Medical and Health Research Ethics, and the study was performed in accordance with the Declaration of Helsinki 2000 and Declaration of Istanbul 2008. All patients included in the analysis gave their written informed consent before their inclusion in the study.

Laboratory Assessment

Serum creatinine concentration was measured by an enzymatic calorimetric method (reagents from Roche Diagnostics, Rotkreutz, Switzerland) IDMS traceable. The coefficient of variation (CV) was 3.7% or less. Serum cystatin C was measured by a turbidimetric immunoassay, traceable to the ERM-DA471/IFCC reference material (reagents from Gentian, Moss, Norway, CV ≤ 5.0%). Both markers were analyzed on Modular P8000 (Roche Diagnostics).

Blood samples for determination of clinical chemistry were drawn in the morning, after fasting (drugs and food) over night. Afterward, GFR was measured by ^{51}CrEDTA clearance in the first cohort. Patients were administered 1.0 mL ^{51}CrEDTA (100 μCi/mL) intravenously (Amersham Int, Kjeller, Norway). Blood samples for determination of ^{51}CrEDTA plasma concentrations were drawn in 7-mL EDTA vacutainers before (0 hour) and then 2, 3, and 4 hours after administration of ^{51}CrEDTA. In patients with an estimated GFR (Cockcroft & Gault-equation, commonly used at our hospital in that period) less than 30 mL/min, an extra sample was also drawn 6 hours after ^{51}CrEDTA administration. GFR was calculated according to the Bröchner Mortensen method.[30] In the iohexol cohort mGFR was determined by iohexol clearance (Omnipaque 300 mg iodine/mL; GE Healthcare) with blood sampling 2 and 5 hours after the iohexol injection. When eGFR with MDRD equation or CKD-EPI$_{creatinine}$ equation was below 40 mL/min per 1.73 m^2, the last blood sample was obtained 8 to 24 hours after iohexol administration. Serum samples were analyzed by a high-performance liquid chromatography system and calculated according to the Bröchner Mortensen method as previously described.[31] The coefficient of variation of our serum method is less than 6%.

Development of a new Combined GFR-Estimating Equation

The statistical analyses were performed by the use of Microsoft Excel (version 2002 SP3), R version 3.3.2 and SPSS Statistics 18. The new formula was constructed by backward and forward multiple linear regression analysis between logarithmically transformed values of mGFR and the following transformed covariates: age, sex, creatinine and cystatin C. The addition of body weight in the multiple linear regression analysis had no significant effect. *P* values for variable inclusion and exclusion were 0.05.

Evaluation of Our New Equation and Comparison of Different Well-Known Equations

Bias was defined as the difference in GFR between the test method (eGFR-equation) and reference method (mGFR), calculated as median absolute differences (mL/min per 1.73 m^2) in GFR. Precision was assessed as the interquartile range (IQR) of the differences. Accuracy was defined by P15 and P30 which are the percentage of test method eGFR results within 15% and 30 % of the reference mGFR (mL/min per 1.73 m^2). Confidence intervals (CI) were calculated by means of bootstraps methods with 1000 replicates. Bias, IQR, and accuracy were also evaluated in relation to 2 mGFR regions; below or above (including) 60 mL/min per 1.73 m^2. All absolute GFR values given in the following paragraphs are in mL/min per 1.73 m^2 and accuracy (P15 and P30) in %. Bland Altman plots were used to compare the different equations.

We calculated eGFR using 4 creatinine-eGFR-equations (CKD-EPI$_{creatinine}$, MDRD, Cockcroft-Gault and Nankivell),[3-6] 2 cystatin-C-eGFR-equations (CKD-EPI $_{cysC\ 2012}$, Caucasian, Asian, pediatric, and adult cohorts (CAPA))[32,33] and 2 combined equations with both creatinine and cystatin C (CKD-EPI$_{creatinine+cys\ C\ 2012}$, new equation)[33] (Table 1). The clearance values from the Cockcroft-Gault and Nankivell formula were standardized to body surface area ad modum DuBois.[34]

External Evaluation in a Recent Cohort Without Trimethoprim (Iohexol Cohort)

We compared the GFR-equations (Table 1) in a patient group that had received trimethoprim 8 weeks posttransplant and the same patients without concomitant trimethoprim 1 year after the transplantation. Bias, IQR, CI, and accuracy 1 year posttransplant were calculated as described in the previous section.

RESULTS

Patient characteristics of the kidney recipients that participated in the study are listed in Table 2, including 297 patients for generating the new equation (reference group), 297 for comparing the equations with ^{51}CrEDTA clearance (test group), and 133 patients for external evaluation (with and without trimethoprim) with iohexol clearance (iohexol cohort). The patient characteristics of the reference group and the test group were almost identical with no significant (statistically and biologically) differences, supporting the validity of the randomization. There were 194 men and 103 women in the test group. The median (2.5-97.5 percentile) age was 52 (23-75) years, cystatin C 1.63 (1.00-3.04) mg/L, creatinine 117 (63-220) μmol/L, and measured GFR 51 (29-78) mL/min per 1.73 m^2.

Comparison of the Equations in the Test Group

Table 3 shows median bias, IQR, and accuracy for the 8 tested equations. These data demonstrate that among the creatinine-based equations, the MDRD equation had the lowest bias both above and below mGFR 60 mL/min per 1.73 m^2 (median bias, 0.70 ± 12.3 and 2.52 ± 16.0, < 60, and ≥ 60 mL/min per 1.73 m^2, respectively) compared to Cockcroft-Gault (8.01 ± 15.4 and 13.5 ± 19.2), CKD-EPI$_{creatinine}$ (3.68 ± 14.6 and 9.85 ± 20.3) and Nankivell (–4.18 ± 13.0 and –24.6 ± 10.1) equations. Also, the MDRD equation was the most accurate of the creatinine equations with P30 of 85% and 87% in the 2 GFR regions. The CKD-EPI$_{creatinine}$ equation showed P30 of 74% and 69%. The Nankivell demonstrated very low accuracy with P30 of 77% and 32%.

Among the cystatin C equations the CKD EPI $_{cystC}$ equation (–9.79 ± 11.5 and –10.2 ± 16.9)) and the CAPA formula (–8.58 ± 10.3 and –11.7 ± 15.6) both underestimated below and above mGFR 60 mL/min per 1.73 m^2. The combined CKD-EPI$_{creatinine+\ cystC}$ equation performed well, especially in the higher GFR-range (–4.56 ± 10.7 and –2.93 ± 14.5), and

TABLE 1.

Equations evaluated in this study

Equation	Formula, mL/min per 1.73 m^2
Creatinine-based	
MDRD	GFR = 30849 × creatinine$^{-1.154}$ × age$^{-0.203}$ × (0.742 for female) × (1.212 for African-Americans)
Cockcroft-Gault[a]	GFR = [(140 – age) × body weight]/(creatinine × 0.815) × (0.85 for female)
CKD-EPI creatinine	GFR = 141 × min(Scr/k, 1)^{a} × max(Scr/k, 1)$^{-1.209}$ × 0.993^{age} × (1.018 for female) × (1.159 for African-Americans), k = 0.7 for female and 0.9 for male, a = –0.329 for female and –0.411 for male
Nankivell formula B[a]	GFR = (6700/creatinine + (body weight (kg)/4) – (urea (mmol/L)/2) – (100/height(m)^2 + (35 for male or 25 for female)
Cystatin C-based	
CAPA	GFR = 130 × (cysC$^{-1.069}$) × (age$^{-0.117}$) – 7
CKD-EPI cyst C	GFR = 133 × min(cys/0.8, 1) $^{-0.499}$ × max(cys/0.8,1)$^{-1.328}$ × 0.996^{age} × 0.932 for female
Combined creatinine and cystatin C	
CKD-EPI creatinine + cyst C	GFR = 135 × min(Scr/k, 1) ^{a} × max(Scr/k, 1)$^{-0.601}$ × min(Scys/0.8, 1)$^{-0.375}$ × max(Scys/0.8,1)$^{-0.711}$ × 0.995^{age} × (0.969 for female) × (1.08 for African Americans), k = 0.7 for female and 0.9 for male, a = –0.248 for female and –0.207 for male
New equation (this study)	GFR = 991.15 × (1.120^{sex})/([age$^{0.097}$] × [cysC$^{0.306}$] × [creatinine$^{0.527}$]), 0 = female, 1 = male

[a] GFR in mL/min.

TABLE 2.

Basic characteristics of the population[a]

Variable	Samples for generating the new equation (reference group)	Samples for comparing the equations (test group)	External evaluation without trimethoprim (iohexol cohort)
Total number	297	297	133
Sex: f/m, n	92/205	103/194	34/99
Age, y	54 (22-76)	52 (23-75)	57 (25-76)
Body weight, kg	76 (50-108)	75 (49-109)	81 (55-118)
Height, cm	175 (153-192)	175 (156-190)	177 (158-191)
Plasma cystatin C, mg/L	1.62 (1.06-3.25)	1.63 (1.00-3.04)	1.47 (0.85-2.31)
Plasma creatinine, μmol/L	115 (65-231)	117 (63-220)	114 (70-191)
Measured GFR, mL/min per 1.73 m^2	53 (28-80)[b]	51 (29-78)[b]	55 (34-89)[c]
mGFR < 60 mL/min per 1.73 m^2, n (%)	207 (70)	219 (74)	78 (59)
mGFR ≥ 60 mL/min per 1.73 m^2, n (%)	90 (30)	78 (26)	55 (41)

[a] Values are given as medians and 2.5th to 97.5th percentiles.

[b] 51CrEDTA clearance.

[c] Iohexol clearance.

F, female; m, male.

the accuracy was good (P30 = 84% and 92%, respectively). The results are also presented in Bland Altman plots (Figure 1A-E), with the bias and its 95% CI.

Evaluation of the New Equation

The new equation eGFR = 991.15 × (1.120^{sex}/([age$^{0.097}$] × [cystatin C$^{0.306}$] × [creatinine$^{0.527}$]); where sex is denoted: 0, female; 1, male exhibited the best accuracy of all the equations with P30 = 91% and 99% and P15 = 73% and 77% and a small bias and IQR (1.19 ± 7.59 and −4.25 ± 10.3).

External Evaluation: 1 Year Posttransplant, Without Trimethoprim (Iohexol Cohort)

There were 34 women and 99 men in this group (Table 2) The median (2.5-97.5 percentile) value at 1 year after transplantation was; age, 57 (25-76) years; cystatin C, 1.47 (0.85-2.31) mg/L; creatinine, 114 (70-191) μmol/L; and mGFR, 55 (34-89) mL/min per 1.73 m^2. The median bias, IQR, and accuracy are presented in Table 3.

Both the new equation and the CKD-EPI$_{creatinine+\ cystC}$ had P30 of 94% and 100%, less than 60 mL/min per 1.73 m^2 and 60 mL/min per 1.73 m^2 or greater, respectively. The new formula had the best P15 of 75% in GFR less than 60, and the combined CKD-EPI$_{creatinine+\ cystC}$ the best P15 of 75% in GFR of 60 mL/min per 1.73 m^2 or greater.

DISCUSSION

The new equation demonstrated a better accuracy with a low bias as well as good precision compared with reference equations. The new equation showed superior validation data in the renal function range below 60 mL/min per 1.73 m^2 which is the most relevant for renal transplant recipients. Our data also confirm that the MDRD equation was the most accurate of the creatinine equations in renal transplant recipients. Trimethoprim, in doses used for prophylaxis in transplantation did not influence the performance of the equations.

A review comparing different equations demonstrated a divergence regarding bias and accuracy between different studies.[2] They differed in methods for measuring GFR and creatinine assay calibration. Another review[35] showed that all studies, except one,[36] reported P30 of creatinine-based equations less than or equal to 80%.[1,23,37-40] Most studies demonstrate that the CKD-EPI$_{creatinine}$ equation is inferior to the MDRD equation in renal transplants,[1,23,36-38,41] as opposed to its performance in populations with chronic kidney disease. Some studies have however documented superiority in kidney transplant recipients.[39,40] A study from 2010 described the development and validation of GFR-estimating equations that incorporate diabetes, transplant, and weight as additional variables together with creatinine, age, sex and race.[42] The addition of the predictor variable "transplant" did not significantly improve equation performance in this study. The MDRD equation performed better than the CKD-EPI$_{creatinine}$ equation in our study.

There are several eGFR equations including cystatin C. One study did not find any advantage of using cystatin C over creatinine; however, the analytic method they used was an ELISA, enzyme-linked immunosorbent assay, which is not commonly recommended when generating different equations.[27] In a review, the performance of different cystatin C-based equations in kidney transplant recipients were evaluated, and they found that cystatin C-based equations showed improvements in accuracy compared with the MDRD equation.[26] Another study examined 670 kidney transplant recipients and found that both the CKD-EPI$_{cyst\ C}$ (mean bias, −2.82; P30 81%) and the combined CKD-EPI$_{creatinine+\ cys\ C}$ (mean bias, −0.54; P30 86%) formula performed better than the CKD-EPI$_{creatinine}$ equation alone.[43]

The CKD-EPI$_{creatinine+cys\ C}$ formula was superior to the CKD-EPI$_{creatinine}$ equation in our cohort, but the CKD-EPI$_{cyst\ C}$ equation was not.

Our study has several strengths. All the patients were included consecutively in a single transplant center, representing the entire Norwegian population because this is the only hospital in Norway performing kidney transplantations. The creatinine and cystatin C measurements were calibrated against a standardized reference method, and measured GFR was performed with ^{51}CrEDTA clearance and iohexol; methods that are in good agreement with the gold standard inulin clearance.[30]

The limitations of the study are that data is from the era when most of our patients used cyclosporine based immunosuppression while tacrolimus is primarily used at present. Both these drugs acutely affect renal hemodynamics and

TABLE 3.

Comparison of the equations

	mGFR with ^{51}CrEDTA 10 weeks posttransplant with trimethoprim		mGFR with iohexol clearance 1 year posttransplant, without trimethoprim	
	mGFR < 60 mL/min per 1.73 m^2 (n = 219)	mGFR ≥ 60 mL/min per 1.73 m^2 (n = 78)	mGFR < 60 mL/min per 1.73 m^2 (n = 78)	mGFR ≥ 60 mL/min per 1.73 m^2 (n = 55)
Median bias (2.5-97.5 CI)				
MDRD	0.70 (–1.17 to 2.19)	2.52 (0.24-6.69)	1.64 (–0.65 to 4.33)	–5.26 (–8.28 to –1.41)
Cockcroft-Gault	8.01 (6.28-9.70)	13.5 (10.6-17.4)	7.01 (3.93-10.7)	7.85 (4.01-12.5)
CKD-EPI creatinine	3.68 (2.70-6.30)	9.85 (6.87-14.3)	3.72 (1.46-7.08)	2.17 (–2.41 to 5.11)
Nankivell formula B	–4.18 (–6.23 to –3.26)	–24.6 (–25.7 to –22.5)	–4.66 (–6.56 to –3.37)	–24.7 (–28.2 to –22.5)
CAPA	–8.58 (–9.78 to –6.98)	–11.7 (–14.5 to –7.58)	–6.64 (–8.76-4.94)	–11.0 (–13.1 to –8.15)
CKD-EPI cyst C	–9.79 (–11.0 to –7.98)	–10.2 (–14.3 to –5.54)	–7.56 (–9.35 to –5.02)	–9.0 (–12.1 to –6.44)
CKD-EPI creatinine + cyst C	–4.56 (–5.75 to –3.23)	–2.93 (–5.29-1.29)	–3.26 (–4.37 to –1.42)	–5.36 (–8.11 to –1.09)
New equation (this study)	1.19 (0.42-2.09)	–4.25 (–6.81 to –2.50)	2.67 (0.98-3.65)	–8.25 (–10.6 to –5.85)
IQR (2.5-97.5 CI)				
MDRD	12.3 (10.2-15.1)	16.0 (11.8-20.9)	11.1 (8.56-14.0)	13.7 (9.54-19.9)
Cockcroft-Gault	15.4 (13.2-17.2)	19.2 (13.4-27.4)	14.7 (10.6-19.3)	16.3 (12.1-21.1)
CKD-EPI creatinine	14.6 (11.9-16.7)	20.3 (14.0-24.1)	13.5 (9.80-18.2)	15.5 (11.0-20.8)
Nankivell formula B	13.0 (11.2-15.2)	10.1 (7.16-13.1)	11.5 (8.30-13.7)	14.7 (9.43-20.0)
CAPA	10.3 (9.0-11.8)	15.6 (12.2-19.2)	10.4 (7.48-12.5)	11.3 (7.68-15.3)
CKD-EPI cyst C	11.5 (9.64-13.1)	16.9 (13.6-20.3)	9.75 (7.82-13.4)	12.0 (8.59-14.7)
CKD-EPI creatinine + cyst C	10.7 (8.89-12.4)	14.5 (11.4-19.1)	8.41 (6.27-10.5)	10.7 (8.61-14.8)
New equation (this study)	7.59 (5.95-9.39)	10.3 (7.73-12.9)	7.80 (5.57-9.58)	8.24 (5.85-12.2)
Accuracy P15 (2.5-97.5 CI), %				
MDRD	58 (52-65)	58 (46-69)	59 (49-70)	56 (42-69)
Cockcroft-Gault	40 (33-46)	35 (23-45)	46 (35-58)	49 (36-62)
CKD-EPI creatinine	50 (43-57)	46 (36-56)	46 (35-56)	64 (51-76)
Nankivell formula B	44 (40-51)	0 (0-0)	53 (41-63)	2 (0-5)
CAPA	37 (31-43)	41 (30-53)	40 (30-51)	49 (36-62)
CKD-EPI cyst C	33 (27-40)	42 (31-54)	36 (25-48)	53 (40-66)
CKD-EPI creatinine + cyst C	51 (44-57)	63 (51-73)	60 (49-70)	75 (64-86)
New equation (this study)	73 (67-79)	77 (67-86)	75 (66-84)	66 (53-78)
Accuracy P30 (2.5-97.5 CI), %				
MDRD	85 (80-89)	87 (80-94)	90 (84-96)	93 (86-98)
Cockcroft-Gault	67 (61-73)	68 (56-78)	70 (60-80)	84 (73-93)
CKD-EPI creatinine	74 (68-80)	69 (59-78)	84 (75-91)	95 (87-100)
Nankivell formula B	77 (68-86)	32 (22-42)	91 (85-96)	31 (18-42)
CAPA	72 (66-78)	77 (67-86)	84 (75-91)	96 (91-100)
CKD-EPI cyst C	69 (63-74)	77 (68-86)	83 (74-90)	98 (95-100)
CKD-EPI creatinine + cyst C	84 (79-89)	92 (86-97)	94 (89-99)	100 (100-100)
New equation (this study)	91 (88-95)	99 (96-100)	94 (88-99)	100 (100-100)

may hence influence the absolute GFR.[44] To our knowledge, there are however no clinically relevant differences between these drugs when it comes to induce renal vasoconstrictive effects.[45] However, in the external evaluation group of more recently transplanted kidney recipients, all patients received tacrolimus and the new formula also performed well in this cohort. We did not measure thyroid hormone status. A thyroid dysfunction can have an effect on cystatin C levels independently of GFR because of its modifying effect of cellular turnover and metabolism. This could ultimately result in increased cystatin C levels in hyperthyroidism and decreased levels in hypothyroidism. Thyroid dysfunction could also affect the glomerulus and kidney perfusion, thus affecting the creatinine levels, but not independently of GFR. Creatinine levels can drop in patients with hyperthyroidism due to the increased renal blood flow and GFR, and the opposite in patients with hypothyroidism.[18] However, clinically, the patients were considered euthyroid. The patients in the ^{51}CrEDTA cohort received trimethoprim-sulfamethoxazole; an antibiotic that could have a mild inhibitory effect on the tubular secretion of creatinine. This is standard prophylaxis in these patients during the first 6 months posttransplant, but the dosage was low; 80 mg trimethoprim. The exact effect of trimethoprim at 80 mg on serum creatinine concentration is not known, whereas the dose 160 mg increases creatinine by approximately 15%.[12] Therefore, in the iohexol cohort we evaluated the formulas against mGFR measured 1 year posttransplant, when trimethoprim had been discontinued in all patients. Trimethoprim did not influence the performance of the equations significantly in this cohort. Corticosteroids in small doses (median dose, 5-10 mg/day) were also administrated. Prednisolone may elevate cystatin C values,[17] but the impact in the current cohort is probably of minimal effect due to the low doses used. Interpretation of the compartment model regarding

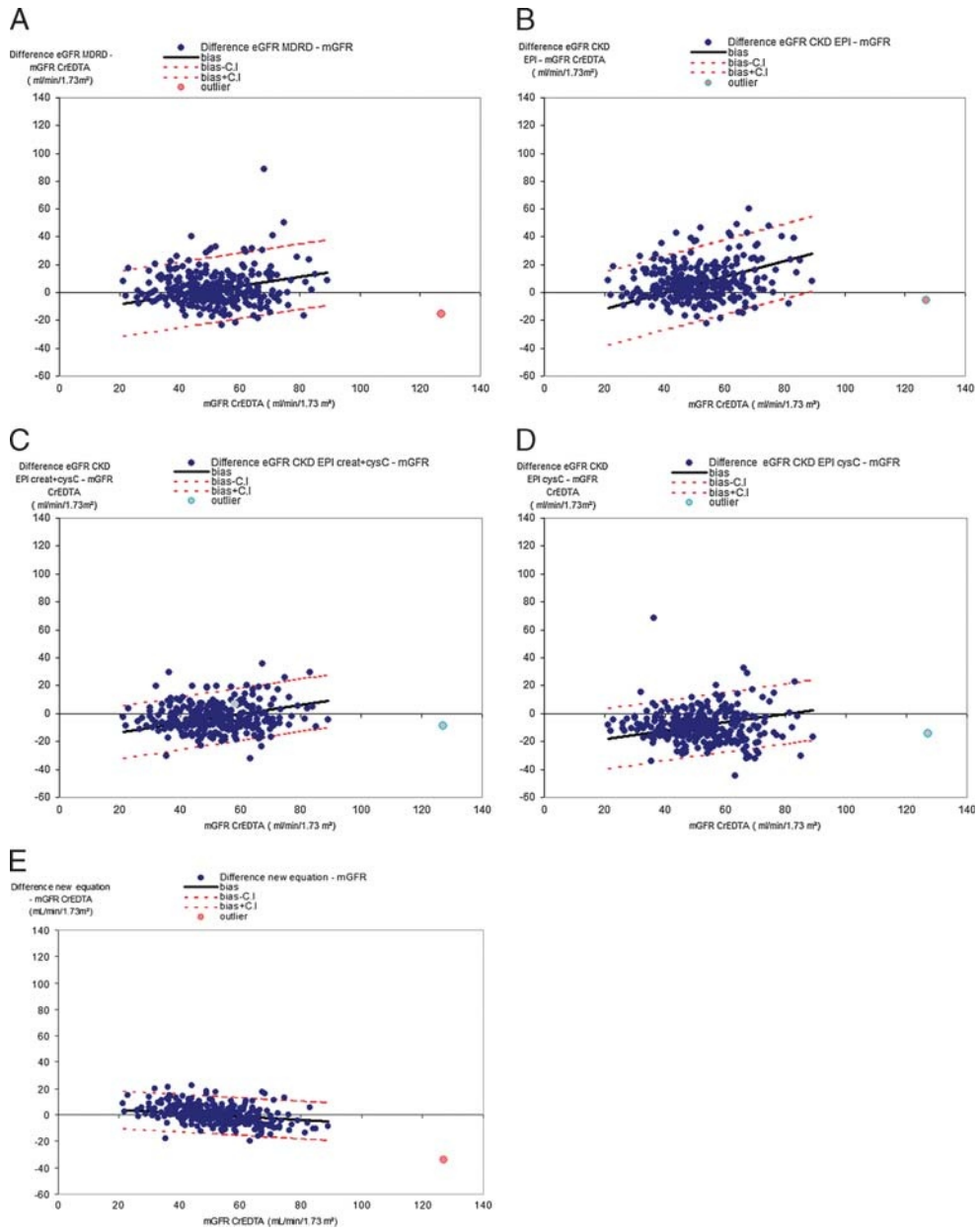

FIGURE 1. A-E, Bland-Altman plots with absolute bias (CI) between eGFR and mGFR for the different equations.

the ^{51}CrEDTA and iohexol methods may be challenged if the patient has edema and also in some patients with impaired graft function with very low GFR if samples are not taken later than 6 hours after ^{51}CrEDTA or iohexol administration.[21] However, there were only few patients with very low GFR and in these cases extended sampling was applied. We do not have detailed information about the race, but the majority of the patients were white. The equations compared in this study are hence without correction factors for Black patients. Finally, the populations used to generate most of the other equations differed from our population. It is just the Nankivell equation that is generated from kidney transplant recipients. The new equation was generated from a cohort of patients in the same institution and laboratory as the ^{51}CrEDTA and iohexol cohort, but the equations were tested both at the timepoint 8 to 10 weeks and 1 year posttransplant, with different mGFR methods and somewhat different immunosuppressive regimens.

In conclusion, the new equation including both creatinine and cystatin C demonstrated the best accuracy of all compared equations, with a low bias as well as very good precision, in the renal function level relevant for renal transplant recipients (below 60 mL/min per 1.73 m^2). The MDRD equation was the most accurate of the creatinine-equations. Trimethoprim in doses used for prophylaxis in transplant recipients did not influence the performance of the equations. Further external validation of the new equation needs to be performed in other kidney transplanted populations outside Norway.

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