Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20-9. DOI: 10.1056/NEJMoa1114248.

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Supplemental Methods

Hypothesis

We hypothesized that equations developed in a diverse dataset would be less biased than our prior equations developed in CKD populations[1,2], especially at higher GFR, and that an equation using creatinine and cystatin C together would be more precise than equations using either filtration marker alone.

Detailed Methods for Equation Development and Validation

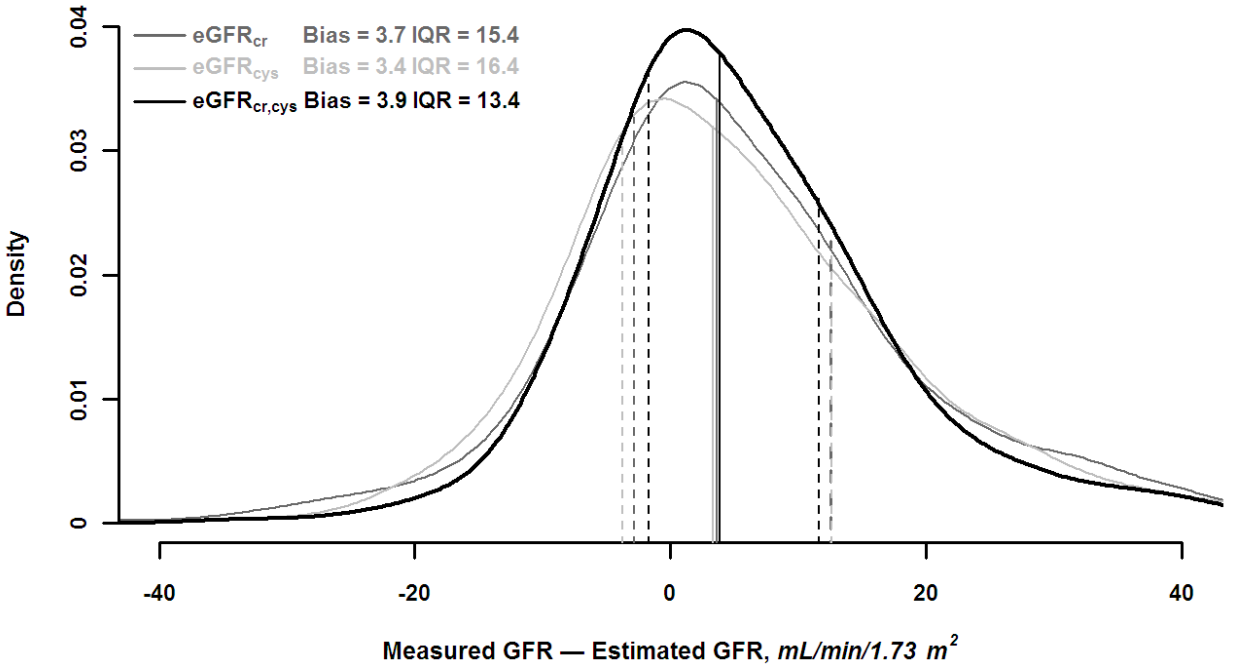
Analyses in the development dataset. As in previous work, we pre-specified a process for developing equations using transformations of continuous variables and inclusion of categorical variables and interactions among all variables to develop a number of candidate equations[3]. The base models were developed using least squares linear regression to relate log transformed measured GFR to log serum creatinine and/or log cystatin C, age, and sex. We also used nonparametric smoothing splines to characterize the shape of the relationship of log measured GFR with log creatinine and log cystatin C. We then approximated the smoothing splines by piecewise linear splines to represent observed non-linearity. Other candidate variables included the other filtration marker, race (black vs. white and other), diabetes (yes/no) and weight. Black race and diabetes status were ascertained in the original studies[4-14]. These additional variables as well as pair-wise interactions among all variables were included if they were significant at a p-value of <0.01 for additional variables and <0.001 for interactions. Models that demonstrated improved model performance [relative reduction in root mean square error (RMSE) by 2% or more overall and 5% in pre-specified subgroups of estimated GFR, age, sex, race, body mass index (BMI) and diabetes] were brought forward into internal validation. We examined

heterogeneity among studies by cross-validation, comparing coefficients for Black and diabetes within each study that included such patients, and examination of relative performance of the equations among studies. We also evaluated performance by level of proteinuria in the subset of studies with available data.

Analyses in the internal validation dataset. We verified the statistical significance of predictor variables and interactions in all models[3]. Models that met these criteria were brought forward into external validation. Development and internal validation datasets were combined into one dataset (called "development dataset" hereafter) to derive final coefficients for each model.

Analyses in the external validation dataset. As in our prior work, we used a pre-specified process to compare performance of the multiple models developed in the development dataset to each other as well as to the CKD-EPI creatinine equation[3], and to our prior equations using cystatin C developed in CKD populations re-expressed for standardized cystatin C values[1,2] (Table S3). We also compared the predictions from the creatinine-cystatin C equation to those calculated from the average of the CKD-EPI creatinine equation and cystatin C equation. We compared performance of equations in the overall dataset and in the subgroups described above, and final models were selected based on the combination of ranking of RMSE overall and within subgroups, clinically significant differences, as well as ease of application in clinical practice.[3] For all steps, sensitivity analyses evaluated robustness of results across studies.

Figure S1: Distribution of difference and percent difference between measured and estimated GFR. Top panel: Difference between measured and estimated GFR. Solid lines indicate median difference (median bias) and dashed lines indicate 25th and 75th percentile for the difference (interquartile range). Bottom panel: Percent difference between measured and estimated GFR. Distance between the black dashed lines indicate the proportion that fall within 30% of measured GFR (P_{30}) and distance between the gray dashed lines indicate the proportion that fall within 20% of measured GFR (P_{20}). 1-P_{30} and 1-P_{20} represent the proportion with errors larger than 30% and 20%, respectively.



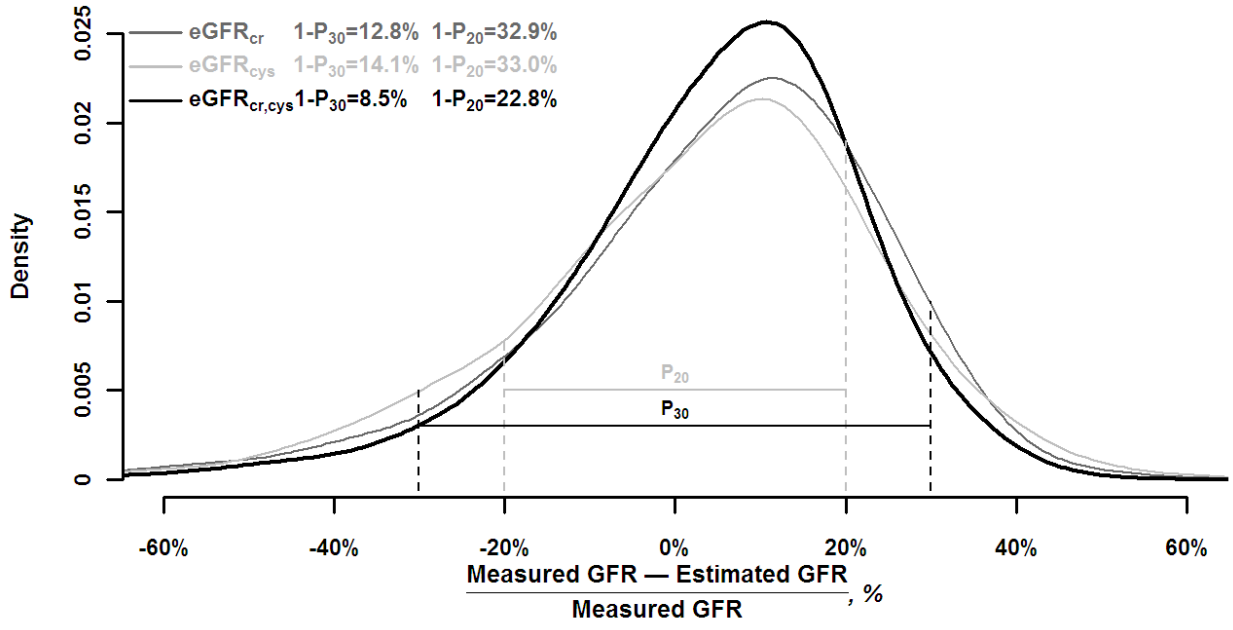


Figure S2: Performance of estimating equations by clinical subgroups. Top panel Bias: Median difference between measured and estimated GFR; Bottom panel: Accuracy: Percentage of estimates greater than 30% of measured GFR ($1\text{-}P_{30}$)

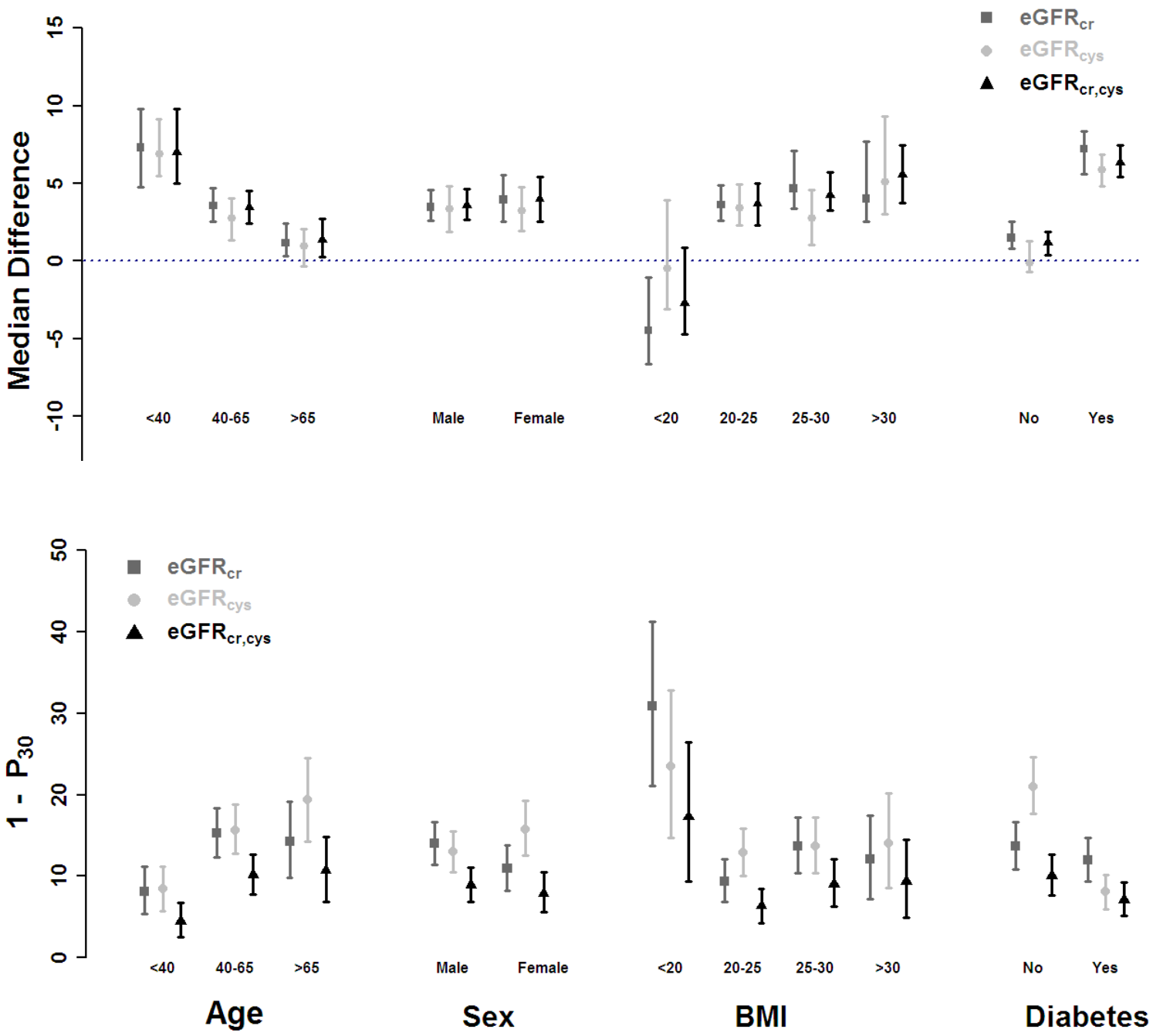


Table S1a. Category 1: Studies and Participant Characteristics

Name	MDRD Study[4]	AASK[5]	DCCT[6]	CSG[7]	CRIC[8]	CCFP	CCFP Donor
Study Characteristics							
Type	RS	RS	RS	RS	RS	CP	CP
Center	MC	MC	MC	MC	MC	SC	SC
N	1046	1647	985	285	653	88	96
Filtration Marker	Iothalamate	Iothalamate	Iothalamate	Iothalamate	Iothalamate	Iothalamate	Iothalamate
Dates	1989-1992	1995-1998	1987-1989	1987-1992	2003-2005	1996-2003	1996-2003
Clinical Characteristics							
Age (years), mean (SD)	52 (13)	54 (10)	29 (6)	34 (8)	55 (14)	54 (13)	43 (12)
Age categories, N (%)							
<40	215 (21)	168 (10)	985 (100)	210 (74)	115 (18)	12 (14)	38 (40)
40-65	660 (63)	1211 (74)	0 (0)	75 (26)	345 (53)	58 (66)	57 (59)
>65	171 (16)	268 (16)	0 (0)	0 (0)	193 (30)	18 (20)	1 (1)
Sex (Female), N (%)	408 (39)	592 (36)	450 (46)	126 (44)	301 (46)	33 (38)	48 (50)
Blacks, N (%)	102 (10)	1647 (100)	27 (3)	23 (8)	283 (43)	7 (8)	10 (10)
Diabetes, N (%)	60 (6)	0 (0)	985 (100)	285 (100)	294 (45)	18 (20)	3 (3)
Type	Type 2	None	Type 1	Type 1	Type 1 and 2	Type 1 and 2	None
Height (cm), mean (SD)	171 (10)	171 (10)	173 (10)	170 (10)	169 (10)	172 (10)	172 (9)
Weight (kg), mean (SD)	79 (16)	90 (21)	73 (12)	74 (13)	91 (24)	85 (21)	78 (15)
BMI (kg/m^2), mean (SD)	27 (4)	31 (7)	25 (3)	26 (6)	32 (8)	28 (6)	26 (4)
<20, N (%)	4 (40)	38 (2)	37 (4)	42 (15)	21(3)	4 (5)	6 (6)
20-25, N (%)	327 (31)	269 (16)	557 (57)	94 (33)	98 (15)	23 (26)	34 (35)
25-30, N (%)	431 (41)	555 (34)	343 (35)	98 (35)	185 (28)	32 (36)	36 (38)
>30, N (%)	248 (24)	785 (48)	48 (5)	50 (18)	349 (53)	29 (33)	20 (21)
GFR ($mL/min/1.73\ m^2$), mean (SD)	33 (14)	57 (23)	124 (20)	72 (33)	50 (21)	53 (31)	102 (18)
Serum creatinine (mg/dL), mean (SD)	2.3 (1.1)	1.7 (0.8)	0.8 (0.1)	1.4 (0.6)	1.7 (0.6)	1.7 (1.1)	0.8 (0.2)
Standardized Cystatin C (mg/dL), mean (SD)	2.1 (0.7)	1.5 (0.6)	0.7 (0.1)	1.3 (0.6)	1.6 (0.5)	1.7 (0.9)	0.8 (0.1)

Table S1a. Category 1: Studies and Participant Characteristics (continued)

Name	MAYO CKD[10]	MAYO Donor[10]	GRONINGEN CKD[11]	GRONINGEN Donor[12]	RASS[13]	CRISP[14]
Study Characteristics						
Type	CP	CP	CP	CP	RS	Cohort
Center	SC	SC	SC	SC	MC	MC
N	203	50	29	34	39	197
Filtration Marker	Iothalamate	Iothalamate	Iothalamate	Iothalamate	Iothalamate, Iohexol	Iothalamate
Dates	1999-2000	1996-2002	2005 - 2007	2005-2007	1998-2006	2000-2001
Clinical Characteristics						
Age (years), mean (SD)	55 (16)	41 (11)	41(14)	52 (13)	24 (5)	34 (8)
Age categories, N (%)						
<40	39 (19)	19 (38)	17 (59)	6 (18)	39 (100)	145 (74)
40-65	103 (51)	30 (60)	12 (41)	22 (65)	0 (0)	52 (26)
>65	61(30)	1 (2)	0 (0)	6 (18)	0 (0)	0 (0)
Sex (Female), N (%)	91 (45)	34 (68)	27 (59)	13 (38)	15 (38)	117 (59)
Blacks, N (%)	1 (0)	0 (0)	1 (3)	0 (0)	1 (3)	21 (11)
Diabetes, N (%)	37 (18)	0 (0)	5 (17)	0 (0)	39 (100)	0 (0)
Type	Type 1 and 2	None	Type 1 and 2	None	Type 1	None
Height (cm), mean (SD)	171 (10)	168 (9)	170 (11)	177 (10)	171 (10)	173 (11)
Weight (kg), mean (SD)	87 (23)	80 (17)	71(14)	83 (17)	75 (15)	78 (18)
BMI (kg/m^2), mean (SD)	30 (7)	28 (6)	24 (3)	26 (4)	26 (4)	26 (5)
<20, N (%)	7 (3)	1 (2)	2 (7)	1 (3)	0 (0)	15 (8)
20-25, N (%)	44 (22)	14 (28)	16 (56)	12 (35)	19 (49)	78 (40)
25-30, N (%)	73 (36)	20 (40)	10 (34)	13 (38)	16 (41)	69 (35)
>30, N (%)	79 (39)	15 (30)	1 (3)	8 (24)	4 (10)	35 (18)
GFR ($mL/min/1.73\ m^2$), mean (SD)	51 (29)	101 (16)	82 (33)	103 (19)	143 (19)	95 (23)
Serum creatinine (mg/dL), mean (SD)	1.6 (0.2)	0.7 (0.2)	1.1 (0.8)	0.9 (0.1)	0.8 (0.1)	0.9 (0.2)
Standardized Cystatin C (mg/dL), mean (SD)	1.8 (0.8)	0.9 (0.1)	1.4 (0.7)	1.0 (0.1)	0.8 (0.1)	0.9 (0.2)

Abbreviations: MDRD Study, Modification of Diet in Renal Disease Study; AASK, African American Study of Kidney Diseases and Hypertension; DCCT, Diabetes Control and Complications Trial; CSG, Collaborative Study Group: Captopril in Diabetic Nephropathy Study; CRIC, Chronic Renal Insufficiency Cohort Study; CCF, Cleveland Clinic Foundation; MC, multicenter; SC, Single Center; RS, research study; CP, clinical population; SC, single center; MC, multi-center; GFR, glomerular filtration rate; BMI, body mass index; SD, standard deviation
To convert GFR from $mL/min/1.73\ m^2$ to $mL/s/m^2$, multiply by 0.0167. To convert serum creatinine from mg/dL to µmol/L, multiply by 88.4.

Table S1b: Category 2: Studies and Participant Characteristics

Name	NephroTest[15]	Steno [16-18]	RASS[13]	Lund CKD[19]	Lund Donor[19]
Study Characteristics					
Type	CP	RCT	RCT	CP	CP
Center	SC	SC	MC	SC	SC
N	313	245	211	343	7
Filtration Marker	EDTA	EDTA	Iohexol	Iohexol	Iohexol
Dates	1993-2007	1989-2003	1998-2006	2003	2003
Clinical Characteristics					
Age (years), mean (SD)	59 (15)	43 (9)	33 (9)	58 (16)	59 (10)
Age categories, N (%)					
<40	38 (12)	110 (45)	161 (76)	46 (13)	2 (29)
40-65	159 (51)	134 (55)	50 (24)	182 (53)	5 (71)
>65	116 (37)	1 (0)	0 (0)	115 (34)	0 (0)
Sex (Female), N (%)	90 (29)	85 (35)	109 (52)	167 (49)	5 (71)
Blacks, N (%)	25 (8)	0 (0)	5 (2)	0 (0)	0 (0)
Diabetes, N (%)	72 (23)	245 (100)	211 (100)	66 (19)	0 (0)
Type	Type 1 and 2	Type 1	Type 1	Type 1 and 2	None
Height (cm), mean (SD)	168 (9)	173 (9)	171 (9)	170 (10)	174 (9)
Weight (kg), mean (SD)	74 (16)	72 (12)	76 (14)	74 (17)	79 (10)
BMI (kg/m^2), mean (SD)	26 (4)	24 (3)	26 (4)	26 (5)	26 (2)
<20, N (%)	22 (7)	20 (8)	0 (0)	39 (11)	0 (0)
20-25, N (%)	117 (37)	147 (60)	98 (46)	139 (41)	2 (29)
25-30, N (%)	119 (38)	66 (27)	84 (40)	112 (33)	5 (71)
>30, N (%)	55 (18)	12 (5)	29 (14)	53 (15)	0 (0)
GFR ($mL/min/1.73\ m^2$), mean (SD)	35 (18)	72 (31)	128 (19)	64 (33)	88 (18)
Serum creatinine (mg/dL), mean (SD)	2.4 (1.2)	1.5 (0.8)	0.8 (0.1)	1.5 (1.1)	1.0 (0.2)
Standardized Cystatin C (mg/dL), mean (SD)	2.0 (0.7)	1.3 (0.6)	0.8 (0.1)	1.6 (0.8)	1.0 (0.2)

RASS, Renin Angiotensin System Study; MC, multicenter; SC, Single Center; CP, clinical population; SC, single center; MC, multi-center; GFR, glomerular filtration rate; BMI, body mass index; SD, standard deviation; EDTA, ethylenediaminetetraacetic acid. To convert GFR from $mL/min/1.73\ m^2$ to $mL/s/m^2$, multiply by 0.0167. To convert serum creatinine from mg/dL to µmol/L, multiply by 88.4.

Table S2: Serum Cystatin C Measurements or Calibration by Study

Study	Date	Sample Size	Instrument	Calibration Equation to IFCC Serum Cystatin C
Direct Measurements at Cleveland Clinic				
MDRD Study[3]	Jul 2004	1,047	CCRL BN-II	IFCC Scys = 1.12 x (0.083 + 0.789 x MDRD Scys)
AASK[6]	May 2005	1,645	CCRL BN-II	IFCC Scys = 1.12 x (0.083 + 0.789 x AASK Scys)
CSG[7]	Apr 2005	386	CCRL BN-II	IFCC Scys = 1.12 x (0.083 + 0.789 x CSG Scys)
NephroTest[15]	Sep 2005	438	CCRL BN-II	IFCC Scys = 1.12 x (0.083 + 0.789 x Paris Scys)
Steno[16-18]	Dec 2005	260	CCRL BN-II	IFCC Scys = 1.12 x (0.083 + 0.789 x Steno Scys)
RASS[13]	Feb 2007	524	CCRL BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x RASS Scys)
NHS	Feb 2007	58	CCRL BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x NHS Scys)
GRONIGEN [11]	Mar 2007	200	CCRL BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x Groningen Scys)
CCF Prospective	Apr 2007	200	CCRL BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x CCF Scys)
CRISP[14]	May 2007	218	CCRL BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x CRISP Scys)
Lund[19]	CCRL: Oct 2005 Lund: Feb-Oct 2003	200	CCRL: BNII Lund: Hitachi Mod P	IFCC Scys = 1.12 x (0.083 + 0.789 x (-0.574 + 1.611 x Grubb/Lund Scys))
DCCT[6]	CCRL: Feb-Mar 2006 DCCT: Feb-Apr 2005	197	CCRL: BNII DCCT: ProSpec	IFCC Scys = 1.12 x (0.083 + 0.789 x (0.018 + 0.882 x DCCT Scys))
CRIC[8]	CCRL: 2003 Penn: Feb 2008	39*	CCRL: BN-II Penn: BN-II	IFCC Scys = 1.12 x (0.083+ 0.789 x (0.039 + 1.061 x CRIC Cys)
MAYO[10]	CCRL: Dec 2006 Mayo: Oct 1999-Mar 2000	292	CCRL: BN-II Mayo: BN-II	IFCC Scys = 1.12 x (0.105 + 0.848 x (0.076 + 1.023 x Mayo Scys))

* Calibration panel measured in triplicate at both CCF and Penn
CCRL, Cleveland Clinic Research Laboratory; BN-II, Dade Behring Nephelometer; Scys, serum cystatin C

Table S3: Previously Developed Equations in CKD Populations Re-expressed for Use with Standardized Serum Creatinine or Serum Cystatin C

MDRD Study equation [3]	175 x standardized Scr $^{-1.154}$ x age^{-203} x 0.75 [if female] x 1.210 [if black]
Cystatin C [1,2]	127.7 * (-0.105 + 1.13 x standardized Scys)$^{-1.17}$ x age$^{-0.13}$ x 0.91 [if female] x 1.06 [if black]
Creatinine-Cystatin C[1,2]	177.6 * Scr$^{-0.65}$ x (-0.105 + 1.13 x standardized Scys)$^{-0.57}$ x age$^{-0.20}$ x 0.82 [if female] x 1.11 [if black]

Scr, serum creatinine; Scys, serum cystatin C. Units for serum creatinine are mg/dl ; for cystatin C are mg/L; and for age is years.

Table S4: Forms of Variables and Coefficients in the CKD-EPI Equations Developed in Diverse Population

Variable	Creatinine		Cystatin C		Creatinine and Cystatin C	
	Form for Estimating GFR on Log Scale	**Coefficient (95% CI) for Estimating GFR on Natural Scale**	**Form for Estimating GFR on Log Scale**	**Coefficient (95% CI) for Estimating GFR on Natural Scale**	**Form for Estimating GFR on Log Scale**	**Coefficient (95% CI) for Estimating GFR on Natural Scale**
Creatinine	2-slope spline on the log scale with sex specific knots (0.7 mg/dl for women and 0.9 mg/dl for men)	Above the knot All: $Scr^{-1.209\ (-1.198,\ -1.220)}$ Below the knot Women: $Scr^{-0.329\ (-0.230,\ -0.428)}$ Men: $Scr^{-0.411\ (-0.314,\ -0.508)}$			2-slope spline on the log scale with sex specific knots (0.7 mg/dl for women and 0.9 mg/dl for men)	Above the knot All: $Scr^{—0.601\ (-0.630,\ -0.571)}$ Below the knot Women: $Scr^{-0.248(-0.364,\ -0.132)}$ Men: $Scr^{-0.207\ (-0.308,-0.107)}$
Cystatin C			2-slope spline on the log scale with knots at 0.8 mg/L	Above the knot $Scys^{-1.328(-1.344,\ -1.312)}$ Below the knot: $Scys^{-0.499\ (-0.610,\ -0.388)}$	2-slope spline on the log scale with knots at 0.8 mg/L	Above the knot $Scys^{-0.711(-0.744,\ -0.678)}$ Below the knot: $Scys^{-0.375(-0.477,\ -0.274)}$
Race	Black vs. White or other	1.159 (1.144, 1.170) if Black 1.0 if White or other			Black vs. White or other	1.08 (1.067, 1.093) if Black 1.0 if White or other
Sex	Female vs. Male	1.018 (1.007, 1.029) if Female 1.0 if Male	Female vs. Male	0.932 (0.921, 0.944) if Female 1.0 if Male	Female vs. Male	0.969 (0.958, 0.980) if Female 1.0 if Male
Age	Linear on the natural scale	$0.993\ (0.9925,\ 0.9933)^{Age}$	Linear on the natural scale	$0.996\ (0.9957,\ 0.9966)^{Age}$	Linear on the natural scale	$0.995\ (0.9948,\ 0.9957)^{Age}$

Formulation of the equations are shown in Table 2

Variables included in the CKD-EPI cystatin C equation are spline log cystatin C, sex and age. Serum cystatin C is modeled as a two-slope linear spline with a knot at 60 nmol/L (0.8 mg/L) and allows for a steeper slope of logarithm of GFR vs. logarithm of cystatin C above the knot [-1.328 (-1.344, -1.312)] and a less steep slope below the knot [0.499 (-0.610, -0.388)]. Females and older adults have lower GFR than males and younger adults (7% lower for men vs. women and 2% lower GFR per 5 years of age for the same level of cystatin C).

Variables included in the CKD-EPI creatinine and cystatin C equation are spline log serum creatinine, spline log serum cystatin C, sex, race and age. Log creatinine is modeled as it is in the CKD-EPI creatinine equation; a two-slope linear spline term with sex-specific knots at 62 µmol/L (0.7 mg/dL) in women and 80 µmol/L (0.9 mg/dL) in men. Log cystatin C is modeled as in the cystatin C equation. Above the knots for log creatinine

and log cystatin C, the slopes are similar to each other [-0.601 (-0.630, -0.571)] and [-0.711(-0.744, -0.678), respectively] and are approximately half the size of the corresponding coefficients in the equations with each marker alone. The relationship between sex and estimated GFR varies according to the level of serum creatinine. The predicted female-to-male ratio in GFR varies from 0.87 to 0.93 when the serum creatinine is between 44 to 71 µmol/L (0.5 and 0.8 mg/dL), and is 0.83 when serum creatinine is $\geq$80 µmol/L ($\geq$0.9 mg/dL). For every additional 5 years of age, there is a 2% lower GFR. The coefficient for Blacks predicts an 8% higher GFR for the same level of creatinine or cystatin C compared to 16% in the CKD-EPI creatinine equation. The addition of diabetes or weight did not improve performance.

To convert serum creatinine from mg/dL to µmol/L, multiply by 88.4. Coefficients for cystatin C and creatinine-cystatin C equations derived from pooled development and internal validation datasets.

Table S5: Newly Developed Equations that May Be of Interest in Research

Spline Log Cystatin C	109 x min(standardized Scys /0.8,1)$^{-0.683}$ x max(standardized Scys /0.8,1)$^{-1.367}$
Spline Log Cystatin C, Age, Sex, Race	132 x min(standardized Scys/0.8,1)$^{-0.491}$ x max(standardized Scys/0.8,1)$^{-1.329}$ x 0.996^{Age} x 0.932 [if female] x 0992 [if black]
Spline Log Cystatin C, Age, Sex, Diabetes	126 x min(standardized Scys/0.8,1)$^{-0.362}$ x max(standardized Scys/0.8,1)$^{-1.318}$ x 0.997^{Age} x 0.934 [if female] x 1.068 [if diabetes]
Spline Log Cystatin C, Age, Sex, Weight	132 x min(standardized Scys/0.8,1)$^{-0.567}$ x max(standardized Scys/0.8,1)$^{-1.329}$ x 0.996^{Age} x 0.949 [if female] x 1.002$^{Weight-80}$

For all of the above equations, min indicates minimum of standardized Scys /0.8 or 1, and max indicates maximum of standardized Scys/08 or 1 .

Units of cystatin C are mg/L, units for age is years and units for weight is kilograms.

Table S6: Comparison of Performance of Equations in the Development Dataset by Level of GFR and Race

Variables included in each equation	Group	Difference (Measured GFR-Estimated GFR) *ml/min/1.73 m²*		% of Estimates greater than 20% or 30% of Measured GFR		RMSE
		Median	IQR	1-P_{20}	1-P_{30}	
Spline Log Creatinine, Age, Sex, Race*	Overall	0.4	14.9	31.8	15.1	0.228
	Estimated GFR					
	>90	2.6	26.4	23.9	9.5	0.196
	60-90	-0.7	20.8	34.4	15.6	0.230
	<60	0.1	9.5	35.3	18.0	0.243
	Race					
	Black	0.0	14.3	34.8	16.3	0.237
	Non-Black	0.6	15.6	29.9	14.3	0.221
Log Cystatin C	Overall	0.2	15.1	33.8	17.2	0.235
	Estimated GFR					
	>90	0.0	26.5	27.3	11.8	0.216
	60-90	1.3	21.9	34.5	18.0	0.235
	<60	0.1	9.9	37.0	19.8	0.244
	Race					
	Black	-0.3	14.1	34.4	18.5	0.242
	Non-Black	0.7	16.2	33.4	16.4	0.230
Spline Log Cystatin C	Overall	0.4	15.0	33.2	16.6	0.232
	Estimated GFR					
	>90	2.7	24.5	24.8	10.5	0.207
	60-90	-1.3	21.1	36.2	18.2	0.234
	<60	0.2	9.8	36.8	19.3	0.245
	Race					
	Black	-0.6	14.3	35.6	19.6	0.245
	Non-Black	1.2	16.0	31.7	14.6	0.223
Spline Log Cystatin C, Age, Sex	Overall	0.3	14.3	31.3	15.1	0.224
	Estimated GFR					
	>90	1.2	24.1	21.9	8.6	0.189
	60-90	-0.5	18.9	31.9	15.4	0.222
	<60	0.4	10.1	36.4	18.7	0.242
	Race					
	Black	0.1	13.1	32.8	17.3	0.235
	Non-Black	0.5	15.3	30.2	13.6	0.216
Spline Log Cystatin C, Age, Sex, Race	Overall	0.3	14.2	31.1	15.1	0.224
	Estimated GFR					
	>90	1.3	23.8	21.8	8.6	0.189
	60-90	-0.6	19.1	31.3	15.2	0.221
	<60	0.4	10.1	36.4	18.9	0.243

	Race					
	Black	0.2	13.1	32.7	17.2	0.235
	Non-Black	0.4	15.3	30.1	13.8	0.216
Spline Log Creatinine, Log Cystatin C, Age, Sex , Race	Overall	0.1	12.1	24.1	9.8	0.195
	Estimated GFR					
	>90	0.4	22.8	17.9	6.3	0.176
	60-90	-0.5	15.5	25.0	10.2	0.194
	<60	0.1	8.0	27.2	11.6	0.206
	Race					
	Black	0.3	11.7	26.2	11.2	0.204
	Non-Black	0.0	12.7	22.7	8.9	0.188

GFR, glomerular filtrate rate; IQR, interquartile range; RMSE, root mean square error
*Similar to CKD-EPI creatinine equation but re-expressed in the development dataset.
Median difference refers to measured GFR – estimated GFR. Interquartile range of the difference refers to the 25-75th percentile. Units of GFR in ml/min/1.73 m^2. To convert GFR from mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.0167.

Table S7: Performance within the Development Dataset with Data Available on Proteinuria, overall and by proteinuria subgroup

Equation	Group	N	Difference (Measured GFR-Estimated GFR) *ml/min/1.73 m²*		% of Estimates greater than 20% or 30% of Measured GFR		RMSE
			Median	IQR	P_{20}	P_{30}	
Creatinine (CKD-EPI,[3])	Overall	4852	0.7	14.6	68.9	85.9	0.223
	Proteinuria < 70 mg/24 hours	2279	2.8	19.7	73.1	89.6	0.206
	Proteinuria > 70 mg/24 hours	2287	-0.6	10.9	65.0	82.5	0.236
Cystatin C	Overall	4852	0.1	13.9	69.6	85.3	0.222
	Proteinuria < 70 mg/24 hours	2279	1.1	18.3	74.4	88.3	0.206
	Proteinuria > 70 mg/24 hours	2287	-0.5	10.7	64.9	82.3	0.238
Creatinine-cystatin C	Overall	4852	0.2	11.9	76.4	90.7	0.193
	Proteinuria < 70 mg/24 hours	2279	0.8	16.4	79.8	92.2	0.182
	Proteinuria > 70 mg/24 hours	2287	-0.1	8.9	73.4	89.2	0.204

GFR, glomerular filtrate rate; IQR, interquartile range; RMSE, root mean square error
Median difference refers to measured GFR – estimated GFR. Interquartile range of the difference refers to the 25-75th percentile. To convert GFR from mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.0167.

Table S8: Performance of Cystatin C Estimating Equation with and without Diabetes in Development and External Validation Datasets

Variables included in each equation	Group	N	Difference (Measured GFR-Estimated GFR) *ml/min/1.73 m²*		% of Estimates greater than 20% or 30% of Measured GFR		RMSE
			Median	IQR	1-P_{20}	1-P_{30}	
Development							
Spline Log Cystatin, Age, Sex	Overall	5352	0.3	14.3	31.3	15.1	0.224
	Estimated GFR						
	>90	1556	1.2	24.1	21.9	8.6	0.189
	60-90	1081	-0.5	18.9	31.9	15.4	0.222
	<60	2715	0.4	10.1	36.4	18.7	0.242
	Diabetes						
	No	3626	-0.6	12.4	34.3	17.5	0.230
	Yes	1726	2.9	18.7	25.0	9.9	0.210
Spline Log Cystatin C, Age, Sex, Diabetes	Overall	5352	0.2	14.3	30.4	14.6	0.222
	Estimated GFR						
	>90	1529	0.1	23.9	20.7	7.8	0.185
	60-90	1100	0.1	19.3	30.8	15.2	0.220
	<60	2723	0.3	9.9	35.7	18.1	0.242
	Diabetes						
	No	3626	0.1	12.4	33.3	16.7	0.229
	Yes	1726	0.4	18.5	24.3	10.1	0.208
External Validation							
Spline Log Cystatin C, Age, Sex	Overall	1119	3.4	16.4	33.0	14.1	0.234
	Estimated GFR						
	>90	320	8.5	22.6	19.4	2.2	0.164
	60-90	229	6.0	19.6	29.3	12.7	0.208
	<60	570	0.4	11.0	42.1	21.4	0.274
	Diabetes						
	No	525	-0.1	15.1	42.3	21.0	0.263
	Yes	594	5.9	16.4	24.7	8.1	0.205
Spline Log Cystatin C, Age, Sex, Diabetes	Overall	1119	1.9	15.7	31.6	14.7	0.231
	Estimated GFR						
	>90	335	7.1	21.9	15.2	2.4	0.152
	60-90	220	4.8	19.7	30..0	12.7	0.206
	<60	564	-0.3	10.3	42.0	22.9	0.275
	Diabetes						
	No	525	0.8	15.1	42.3	21.3	0.262
	Yes	594	3.0	16.0	22.2	8.9	0.199

The addition of diabetes in models with cystatin C led to a small improvement in bias or RMSE in subgroups with diabetes or higher levels of GFR, but given the small number of studies, this

small improvement may not be generalizable, and we concluded not to recommend use of this equation in clinical practice.

GFR, glomerular filtrate rate; IQR, interquartile range; RMSE, root mean square error
Median difference refers to measured GFR – estimated GFR. Interquartile range of the difference refers to the 25-75th percentile. To convert GFR from mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.0167.

Table S9: Performance of Cystatin C Estimating Equation with and without Weight in Development and External Validation Datasets

Variables included in each equation	Group	N	Difference (Measured GFR-Estimated GFR) *ml/min/1.73 m²*		% of Estimates greater than 20% or 30% of Measured GFR		RMSE
			Median	IQR	1-P_{20}	1-P_{30}	
Development							
Spline Log Cystatin C, Age, Sex	Overall	5352	0.3	14.3	31.3	15.1	0.224
	BMI						
	<20	214	-1.5	14.5	36.0	23.8	0.258
	20-25	1585	-0.9	16.0	31.4	14.4	0.220
	25-30	1881	0.1	13.8	28.9	13.9	0.213
	>30	1671	1.5	12.7	33.3	15.9	0.235
Spline Log Cystatin C, Age, Sex, Weight	Overall	5352	0.2	14.2	30.7	14.7	0.222
	BMI						
	<20	214	0.3	15.2	35.5	21.0	0.254
	20-25	1585	0.4	16.0	30.5	13.4	0.219
	25-30	1881	0.2	13.8	27.9	13.8	0.213
	>30	1671	0.1	13.1	33.5	16.2	0.232
External Validation							
Spline Log Cystatin C, Age, Sex	Overall	1119	3.4	16.4	33.0	14.1	0.234
	BMI						
	<20	81	-0.5	15.6	45.7	23.5	0.262
	20-25	503	3.5	15.2	28.0	12.9	0.229
	25-30	386	2.8	16.7	34.2	13.7	0.221
	>30	149	5.2	17.1	39.6	14.1	0.265
Spline Log Cystatin C, Age, Sex, Weight	Overall	1119	3.9	16.3	33.3	13.9	0.235
	BMI						
	<20	81	0.9	16.0	45.7	17.3	0.265
	20-25	503	5.1	15.5	29.2	13.5	0.233
	25-30	386	3.0	16.7	34.2	14.0	0.222
	>30	149	4.3	17.1	38.3	13.4	0.256

GFR, glomerular filtrate rate; IQR, interquartile range; RMSE, root mean square error; BMI, body mass index. Units of BMI are kg/m^2

Median difference refers to measured GFR – estimated GFR. Interquartile range of the difference refers to the 25-75th percentile. To convert GFR from mL/min/1.73 m^2 to mL/s/1.73 m^2, multiply by 0.0167.

Table S10: Performance of Equations Developed in CKD Populations in the External Validation Dataset

Description	Overall	Estimated GFR *ml/min per 1.73 m²*		
		<60	60-89	≥90
Bias, Median Difference (95% CI)				
Creatinine (MDRD Study[4])	6.3 (5.4 - 7.8)	3.3 (2.4 - 4.2)	15.1 (11.9 - 19.4)	17.2 (13.0 - 20.4)
Cystatin C [1,2]	6.0 (4.9 - 7.1)	2.0 (0.8 - 3.1)	12.4 (9.6 - 14.9)	16.0 (12.7 - 17.8)
Creatinine-cystatin C[1,2]	4.9 (4.2 - 5.9)	2.0 (1.3 - 2.9)	10.7 (7.7 - 13.0)	13.5 (9.7 - 16.0)
Precision, IQR of the Difference (95% CI)				
Creatinine (MDRD Study[4])	19.4 (17.4 - 21.1)	11.4 (10.3 - 12.4)	22.8 (20.5 - 27.9)	27.7(24.3 - 34.5)
Cystatin C [1,2]	18.7 (17.5 - 20.0)	13.1 (11.9 - 14.3)	21.1 (17.6 - 24.0)	25.1 (21.4 - 26.9)
Creatinine-cystatin C[1 2]	15.3 (14.0 - 16.3)	9.2 (8.3 - 9.9)	15.0 (13.3 - 17.6)	23.3 (19.9 - 26.4)
Accuracy, Percentage of Estimates Different from Measured GFR by More than 30% (1-P_{30}) (95% CI)				
Creatinine (MDRD Study[4])	17.4 (15.2 - 19.7)	17.9 (15.0 - 20.9)	22.0 (17.2 - 27.1)	10.7 (6.8 - 14.8)
Cystatin C[1 2]	15.8 (13.8 - 18.0)	21.8 (18.7 - 25.3)	10.6 (6.8 - 14.8)	6.4 (3.7 - 9.5)
Creatinine-cystatin C[1,2]	8.1 (6.6 - 9.8)	11.1 (8.6 - 13.7)	5.6 (2.9 - 8.7)	4.2 (2.1 - 6.7)
Accuracy, Percentage of Estimates Different from Measured GFR by More than 20% (1-P_{20}) (95% CI)				
Creatinine (MDRD Study[4])	43.4 (40.5 - 46.5)	41.9 (37.9 - 45.9)	50.2 (44.0 - 56.3)	39.5 (33.3 - 46.3)
Cystatin C[1 2]	38.6 (35.8 - 41.6)	42.8 (38.9 - 46.8)	37.9 (31.8 - 43.8)	29.4 (23.7 - 35.0)
Creatinine-cystatin C[1,2]	27.3 (24.8 - 30.0)	30.5 (26.9 - 34.2)	26.6 (21.3 - 32.6)	21.5 (16.6 - 26.5)

See Table S3 for equations developed in the CKD populations

Table S11: Performance by Study in the Validation Dataset

Equation	Study	N	P_{30}	RMSE	Within Study % Change vs. $eGFR_{Cr,Cys}$	
					P_{30}	RMSE
Creatinine (CKD-EPI,[3])	NephroTest[15]	313	84.3	0.235	-4.0%	-17.4%
	Steno[16-18]	245	86.1	0.231	-7.9%	-23.3%
	RASS[13]	211	95.7	0.188	-3.3%	-15.8%
	Grubb[19]	350	85.4	0.224	-3.9%	-15.0%
Cystatin C	NephroTest[15]	313	76.4	0.257	-13.1%	-28.7%
	Steno[16-18]	245	94.7	0.198	1.3%	-5.5%
	RASS[13]	211	97.6	0.175	-1.4%	-7.5%
	Grubb[19]	350	81.1	0.265	-8.7%	-36.0%
Creatinine-cystatin C	NephroTest[15]	313	87.9	0.200	ref	ref
	Steno[16-18]	245	93.5	0.188	ref	ref
	RASS[13]	211	99.1	0.162	ref	ref
	Grubb[19]	350	88.9	0.194	ref	ref

Dark gray shading indicates that the P_{30} or RMSE for the creatinine-cystatin C equation is 5% or better than the creatinine or cystatin C equations

Light gray shading indicates that the P_{30} or RMSE for the creatinine-cystatin C equation is 0.1-5% better than the creatinine or cystatin C equations

RMSE, root mean square error; P_{30}, percentage of estimates within 30% of measured GFR; RASS, RASS, Renin Angiotensin System Study;

Table S12: Reclassificationof People with Measured GFR of Greater and Lower than 60 ml/min per 1.73 m^2 Using Estimated GFR Computed from the Creatinine to the Creatinine-Cystatin C equation across Subgroups in the Validation Dataset

Group	Number (percent)	mGFR < 60 ml/min/1.73 m^2 Number (percent)			mGFR ≥ 60 ml/min/1.73 m^2 Number (percent)			NRI (95% CI), p-value
		Correct *eGFRcr≥60 and eGFRcr-cys<60*	Incorrect *eGFRcr<60 and eGFRcr-cys≥60*	Net	Correct *eGFRcr<60 and eGFRcr-cys≥60*	Incorrect *eGFRcr≥60 and eGFRcr-cys<60*	Net	
Age								
< 40	357 (32)	3 (4.1)	2 (2.7)	1.4	9 (3.2)	2 (0.7)	2.5	3.8 (-2.5-10.2) 0.24
40-65	530 (47)	8 (2.9)	5 (1.8)	1.1	17 (6.6)	4 (1.6)	5.0	6.1 (1.8-10.5) 0.01
>65	232 (21)	6 (3.2)	1 (0.5)	2.7	2 (4.4)	3 (6.7)	-2.2	0.5 (-9.7-10.6) 0.93
Sex								
Female	456 (41)	5 (2.6)	4 (2.0)	0.5	10 (3.8)	5 (1.9)	1.9	2.4 (-1.8-6.6) 0.26
Male	663 (59)	12 (3.6)	4 (1.2)	2.4	18 (5.5)	4 (1.2)	4.3	6.7 (3.0-10.3) <0.001
Diabetes								
Yes	594 (53)	7 (3.4)	1 (0.5)	2.9	11 (2.8)	4 (1.0)	1.8	4.7 (1.4-8.1) 0.01
No	525 (47)	10 (3.0)	7 (2.1)	0.9	17 (8.6)	5 (2.5)	6.1	7.0 (1.7-12.3) 0.01
BMI								
< 20	81 (7)	6 (13.0)	3 (6.5)	6.5	1 (2.9)	1 (2.9)	0.0	6.5 (-8.5-21.6) 0.40
20-25	503 (45)	8 (3.7)	3 (1.4)	2.3	11 (3.8)	6 (2.1)	1.7	4.1 (-0.1-8.2) 0.05
25-30	386 (35)	3 (1.6)	2 (1.1)	0.5	13 (6.6)	2 (1.0)	5.6	6.1 (1.6-10.6) 0.01
>30	149 (13)	0 (0.0)	0 (0.0)	0.0	3 (4.6)	0 (0.0)	4.6	4.6 (-1.0-9.7) 0.08

mGFR, measured GFR. eGFRcr, estimated GFR from creatinine; eGFRcr-cys, estimated GFR from creatinine and cystatin C; NRI, net reclassification index; BMI, body mass index. Units of age are years and units of BMI are kg/m^2.

Table S13: Reclassification of Measured GFR Above and Below Different Thresholds Using Estimated GFR Computed from the Creatinine to the Creatinine-Cystatin C equation in the Validation Dataset

Measured GFR Threshold, *ml/min/1.73 m²*	**Number (percent)**	**mGFR < threshold ml/min/1.73 m² Number (percent)**			**mGFR ≥ threshold ml/min/1.73 m² Number (percent)**			**NRI (95% CI) p-value**
		Correct *eGFRcr ≥ threshold and eGFRcr-cys < threshold*	**Incorrect** *eGFRcr < threshold and eGFRcr-cys ≥ threshold*	**Net**	**Correct** *eGFR crr< threshold and eGFR cr-cys ≥threshold*	**Incorrect** *eGFRcr ≥ threshold and eGFRcr-cys < threshold*	**Net**	
>90	1119 (100)	21 (2.8)	6 (0.8)	2.0	27 (7.3)	24 (6.5)	0.8	2.8 (-1.2-6.8) 0.17
75	1119 (100)	16 (2.5)	5 (0.8)	1.7	25 (5.3)	18 (3.8)	1.5	3.2 (0-6.3) 0.04
45	1119 (100)	16 (4.0)	5 (1.2)	2.7	12 (1.7)	13 (1.8)	-0.1	2.6 (0-5.2) 0.05
30	1119 (100)	12 (5.5)	2 (0.9)	4.6	17 (1.9)	14 (1.6)	0.3	4.9 (1.4-8.5) 0.01
<15	1119 (100)	2 (3.9)	1 (2.0)	2.0	8 (0.7)	2 (0.2)	0.6	2.5 (-4.2-9.2) 0.46

mGFR, measured GFR. eGFRcr, estimated GFR from creatinine; eGFRcr-cys, estimated GFR from creatinine and cystatin C; NRI, net reclassification index.

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