Estimating residual kidney function in dialysis patients without urine collection

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Residual kidney function contributes substantially to solute clearance in dialysis patients but cannot be assessed without urine collection. We used serum filtration markers to develop dialysis-specific equations to estimate urinary urea clearance without the need for urine collection. In our development cohort, we measured 24-hour urine clearances under close supervision in 44 patients and validated these equations in 826 patients from the Netherlands Cooperative Study on the Adequacy of Dialysis. For the development and validation cohorts, median urinary urea clearance was 2.6 and 2.4 ml/min, respectively. During the 24-hour visit in the development cohort, serum β-trace protein concentrations remained in steady state but concentrations of all other markers increased. In the validation cohort, bias (median measured minus estimated clearance) was low for all equations. Precision was significantly better for β-trace protein and β2-microglobulin equations and the accuracy was significantly greater for β-trace protein, β2-microglobulin, and cystatin C equations, compared with the urea plus creatinine equation. Area under the receiver operator characteristic curve for detecting measured urinary urea clearance by equation-estimated urinary urea clearance (both 2 ml/min or more) were 0.821, 0.850, and 0.796 for β-trace protein, β2-microglobulin, and cystatin C equations, respectively; significantly greater than the 0.663 for the urea plus creatinine equation. Thus, residual renal function can be estimated in dialysis patients without urine collections.

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Residual kidney function (RKF) is associated with improved survival in dialysis patients.[1–4] Even at the low levels of glomerular filtration rate (GFR) in dialysis patients, RKF is a major contributor to solute and volume clearance.[5–7] Dialysis patients with preserved RKF also have lower concentrations of uremic toxins, less volume overload, lower left ventricular mass, less inflammation, lower requirements for erythropoietin, and better quality of life.[4,8,9] Consequently, loss of RKF after starting dialysis is associated with increased risk of death.[10]

RKF is generally expressed as urinary clearance of urea (CL_{UREA}) or the average of urea and creatinine ($CL_{UREA, CREAT}$). Current guidelines recommend assessment of RKF at regular intervals for adjustment of the dialysis prescription and including CL_{UREA} in hemodialysis adequacy if it is ≥2 ml/min.[7,11,12] However, there are no simple methods for assessing RKF that are similar to GFR estimation from serum creatinine in nondialysis patients. In clinical practice, RKF is assessed by timed 24- to 48-hour urine collection with calculation of urea and creatinine clearance.[7] Urine collections, however, are cumbersome for the patients and the dialysis unit staff and prone to errors leading to overestimation or underestimation of RKF.[7] Serum concentrations of low molecular weight proteins, such as β-trace protein (BTP), β2-microglobulin (B2M), and cystatin C are highly correlated with measured GFR.[13–16] Hemodialysis clearance during conventional (diffusive) high-flux hemodialysis is minimal for BTP (~25,000 Da)[17–19] and partial for B2M (11,600 Da)[18,20] and cystatin C (13,300 Da).[18,21] Peritoneal dialysis clearance of B2M and cystatin C is lower than that of urea and creatinine,[22–26] whereas that of BTP has not been reported. High correlation with measured GFR and low or no removal by dialysis makes these markers attractive candidates for assessment of RKF.

The goal of our study was to use serum endogenous filtration markers to develop dialysis-specific equations to assess RKF and replace timed-urine collections. We developed these equations in a cohort of dialysis patients in Baltimore, Maryland, that underwent careful, closely supervised and

monitored 24-hour urine clearance measurements designed to minimize measurement error. We then validated the equations in an external cohort, the NECOSAD (Netherland Cooperative Study on Adequacy of Dialysis).

RESULTS

Clinical characteristics

In the development cohort (RKF Study; $n = 44$), mean age was 55 years, 64% were male and 21% white (Table 1). None of the patients were vegetarian or had undergone limb amputation. Urinary clearance measurements in the RKF Study were performed on an interdialytic day with the following distribution of the study visit days: Monday, 8 (13.1%); Tuesday, 23 (37.7%); Wednesday, 11 (18%); Thursday, 15 (24.6%); Friday, 2 (3.3%); and Sunday, 2 (3.3%). Patients in the validation cohort (NECOSAD; $n = 826$) were older and more likely to be white. In the development and validation cohorts, median 24-hour urine volume was 799 ml and 720 ml, median CL_{UREA} was 2.6 and 2.4 ml/min, and median $CL_{UREA,\ CREAT}$ was 3.1 and 3.2 ml/min/1.73 m^2, respectively.

RKF and serum concentrations of endogenous filtration markers

In the development cohort with serial measurements of serum markers over 24 hours ($n = 44$ patients with 61 visits), the rate of increase in markers was as follows: urea 10.8 mg/dl/day (95% confidence interval [CI]: 8.1 to 13.5; $P < 0.001$), creatinine 1.3 mg/dl/day (95% CI: 0.9–1.7; $P < 0.001$), BTP 0.09 mg/l/day (95% CI: -0.40 to 0.58; $P = 0.71$), B2M 1.27 mg/l/day (95% CI: 0.01–2.53; $P = 0.05$), and cystatin C 0.30 mg/l/day (95% CI: 0.09–0.52; $P = 0.005$). In both cohorts, filtration markers were negatively correlated with CL_{UREA} (or $CL_{UREA,\ CREAT}$) and positively correlated with each other (Figure 1, Figure S1, and Table S1). BTP, B2M, and cystatin C were highly correlated with each other with the highest correlation between BTP and B2M (RKF Study, 0.807; NECOSAD, 0.759).

In the RKF Study, the concentrations of BTP, B2M, and cystatin C were similar in patients with ($n = 5$) or without a history of liver failure or hepatitis. In a subset of NECOSAD patients with previously measured C-reactive protein ($n = 543$), there was no association between C-reactive protein and endogenous filtration markers (Table S2).

Equation development in RKF Study

Using a prespecified variable selection procedure for equation development (see Materials and Methods), we found coefficients for sex to be significant in the models with BTP and B2M estimating CL_{UREA} or $CL_{UREA,\ CREAT}$ (Table S3). In models that included all 3 low molecular weight proteins, BTP, B2M, and cystatin C, the coefficients for BTP and B2M became smaller and coefficients for cystatin C were no longer significant. Forced addition of age, sex, and race to all models, or excluding patients treated with peritoneal dialysis, minimally changed the values of the markers' coefficients and did not improve estimation (Table S4). Based on these data, we selected the parsimonious equations (without forced variables) presented in Table 2 for testing in the external validation cohort.

Equation performance in NECOSAD

Estimating CL_{UREA}. CL_{UREA} estimation using RKF Study equations had low bias for all equations (Table 3). In general, all equations underestimated CL_{UREA} and $CL_{UREA,\ CREAT}$ (Figure 2). Bias was higher (underestimation of measured CL_{UREA}) using BTP, B2M, and cystatin C equations compared with the urea + creatinine equation. However, precision and accuracy were better using BTP, B2M, and BTP + B2M equations compared with the urea + creatinine equation. The combined BTP + B2M equation had the highest precision (lowest interquartile range [IQR]). Bias was lower and accuracy was higher in patients treated with hemodialysis compared with those treated with peritoneal dialysis (Table S5). The diagnostic accuracy for detecting measured $CL_{UREA} \geq 2$ ml/min by equation-estimated $CL_{UREA} \geq 2$ ml/min was significantly higher for BTP, B2M, and cystatin C equations compared with the urea + creatinine equation ($P < 0.001$) (Figure 3, Table S6).

Estimating $CL_{UREA,\ CREAT}$. Compared with previously published equations in nondialysis patients, the RKF Study equations estimating $CL_{UREA,\ CREAT}$ had significantly lower bias ($P < 0.001$), higher accuracy ($P < 0.001$) but similar precision (Table S7). The Hoek cystatin C equation, developed in NECOSAD, overestimated $CL_{UREA,\ CREAT}$ (as compared to underestimation by the RKF Study equation) but had similar precision and accuracy compared with the RKF Study cystatin C equation.

Repeat measurements. There were 162 repeat measurements over a median of 9.1 months (IQR: 8.9, 9.3). The median (IQR) change in CL_{UREA} was −0.7 ml/min (−1.4, −0.01) and in $CL_{UREA,\ CREAT}$ was −1.1 ml/min/1.73 m^2 (−1.9, −0.05). The decline in RKF over time was associated with increase in serum concentrations of filtration markers (Table S8, Figure S2). There was moderate correlation between the initial and repeat clearance measurements and estimations, although the estimating equations underestimated the change in measured clearances over time (Table S9).

DISCUSSION

In this report, we present dialysis-specific equations to estimate CL_{UREA} and $CL_{UREA,\ CREAT}$ using serum filtration markers. These equations do not require timed-urine collection. We developed these equations in a cohort of dialysis patients in Baltimore, Maryland (RKF Study), with carefully monitored urine clearance measurements and then validated them in an external cohort of dialysis patients in the Netherlands (NECOSAD). The low molecular weight protein (BTP, B2M, and cystatin C) equations had better performance than those including metabolites (urea, creatinine). BTP, B2M, and cystatin C equations also had high diagnostic accuracy for identifying patients with $CL_{UREA} \geq 2$ ml/min, the

Table 1 | Clinical characteristics of the patients in the RKF Study and the NECOSAD

Characteristics	Development cohort RKF Study, Baltimore, Maryland, USA	Validation cohort NECOSAD, the Netherlands					
		All participants		Hemodialysis		Peritoneal dialysis	
	Mean ± SD or n (%)	Mean ± SD or n (%)	*P* (vs. RKF Study)	Mean ± SD or n (%)	*P* (vs. RKF Study)	Mean ± SD or n (%)	*P* (vs. RKF Study)
Number	44	826		587		239	
Demographics							
Age, yr	55.43 ± 11.29	60.22 ± 14.45	0.03	63.44 ± 13.33	<0.001	52.28 ± 14.03	0.16
Male	28 (64)	496 (60)	0.75	334 (57)	0.43	162 (67.78)	0.60
White	9 (21)	724 (88)	<0.001	539 (91)	<0.001	185 (77.41)	<0.001
Clinical characteristics							
Cause of ESRD			0.10		0.08		0.04
Diabetes	12 (27)	128 (16)		96 (16)		32 (13)	
Glomerulonephritis	6 (14)	118 (14)		60 (10)		58 (24)	
Other	26 (59)	580 (70)		431 (73)		149 (62)	
Diabetes	23 (52)	174 (21)	<0.001	137 (24)	<0.001	37 (16)	<0.001
Hemodialysis	40 (91)	587 (71)	0.003	587 (100)	<0.001	0 (0)	<0.001
Duration of prior dialysis, mo	26.7 ± 29.0	6.73 ± 4.49	<0.001	5.42 ± 3.96	<0.001	9.96 ± 4.07	<0.001
Height, cm	171.6 ± 10.1	171.3 ± 9.9	0.83	170.4 ± 9.8	0.42	173.6 ± 9.8	0.23
Weight, kg	93.00 ± 27.46	73.81 ± 14.21	<0.001	72.50 ± 14.02	<0.001	77.04 ± 14.20	<0.001
Body mass index, kg/m^2	31.27 ± 7.76	25.10 ± 4.14	<0.001	24.92 ± 4.17	<0.001	25.54 ± 4.05	<0.001
Body surface area, m^2	2.04 ± 0.31	1.86 ± 0.20	<0.001	1.83 ± 0.20	<0.001	1.91 ± 0.20	<0.001
Urea volume of distribution, l	44.9 ± 11.6	38.0 ± 6.7	<0.001	37.1 ± 6.4	<0.001	40.1 ± 7.0	<0.001
24-h urine volume, ml, median (IQR)	799 (319, 1154)	720 (389, 1217)	0.33	679.71 (350, 1097)	0.75	889 (472, 1522)	0.02
Urine volume ≥250 ml	34 (77)	718 (87)	0.07	501 (85)	0.19	217 (91)	0.02
Measured urinary clearances, median (IQR)							
Urea clearance, ml/min	2.59 (0.92, 4.07)	2.43 (1.31, 3.94)	0.84	2.37 (1.28, 3.81)	0.92	2.59 (1.41, 4.48)	0.40
Urea clearance, ml/min/1.73 m^2	2.42 (0.70, 3.31)	2.30 (1.23, 3.66)	0.24	2.24 (1.21, 3.57)	0.33	2.36 (1.30, 3.97)	0.13
Creatinine clearance, ml/min	4.32 (1.62, 7.12)	4.24 (2.33, 6.97)	0.48	4.14 (2.21, 6.87)	0.37	4.51 (2.73, 7.57)	0.83
Creatinine clearance, ml/min/1.73 m^2	3.71 (1.35, 5.96)	4.03 (2.19, 6.57)	0.61	3.98 (2.11, 6.52)	0.68	4.05 (2.39, 7.09)	0.48
Average of urea and creatinine clearance, ml/min	3.58 (1.36, 5.50)	3.42 (1.91, 5.42)	0.67	3.38 (1.77, 5.19)	0.51	3.55 (2.13, 6.11)	0.88
Average of urea and creatinine clearance, ml/min/1.73 m^2	3.09 (1.05, 4.82)	3.17 (1.77, 5.13)	0.45	3.14 (1.71, 4.95)	0.54	3.24 (1.95, 5.62)	0.30
Weekly renal standard Kt/V_{UREA}	0.58 (0.16, 0.90)	0.65 (0.36, 1.06)	0.11	0.65 (0.35, 1.04)	0.13	0.65 (0.37, 1.12)	0.08
Weekly renal standard Kt/V_{CREAT}	1.01 (0.33, 1.73)	1.15 (0.62, 1.92)	0.27	1.15 (0.60, 1.90)	0.29	1.15 (0.67, 1.94)	0.282
Weekly renal standard Kt/$V_{UREA, CREAT}$	0.83 (0.25, 1.47)	0.90 (0.50, 1.50)	0.19	0.90 (0.49, 1.48)	0.21	0.88 (0.53, 1.56)	0.18
Endogenous serum filtration markers							
Urea nitrogen,[a] mg/dl	52.50 (15.74)	64.92 (17.09)	<0.001	66.91 (16.67)	<0.001	60.05 (17.17)	0.007
Creatinine,[b] mg/dl	8.57 (3.07)	8.73 (2.77)	0.70	8.53 (2.63)	0.93	9.22 (3.03)	0.19
β-Trace protein, mg/l	7.36 (3.13)	6.93 (2.58)	0.28	6.77 (2.45)	0.13	7.32 (2.86)	0.93
β2 Microglobulin, mg/l	24.28 (9.50)	25.5 (9.49)	0.42	25.55 (9.56)	0.40	25.23 (9.34)	0.53
Cystatin C, mg/l	5.24 (1.26)	5.07 (1.10)	0.35	5.02 (1.07)	0.20	5.21 (1.17)	0.91

ESRD, end-stage renal disease; IQR, interquartile range (25th, 75th percentiles); Kt/V, weekly clearance (Kt) divided by volume of total body water (V); NECOSAD, The Netherlands Cooperative Study on the Adequacy of Dialysis; RKF, Residual Kidney Function.
Conversion factors for units:
[a]urea nitrogen in mg/dl to mmol/l, 0.357.
[b]creatinine in mg/dl to μmol/l, × 88.4.

KDOQI (Kidney Disease Outcomes Quality Initiative) threshold for considering urinary CL_{UREA} in hemodialysis adequacy. These equations are valid for use in dialysis patients with self-reported urine volume ≥1 cup/day and could be considered for use in place of urine collections.

BTP is a 168 amino acid glycoprotein with varying molecular weight between 23,000 and 29,000 Da.[13] The major sources of circulating BTP are leptomeninges, arachnoid cells, choroid plexus, and oligodendrocytes of the central nervous system.[13] Serum BTP can be used to estimate GFR in non-dialysis patients.[15,27,28] BTP is not removed by conventional low- or high-flux dialysis.[17,19] BTP clearance by peritoneal dialysis has not been reported. In our development cohort, serum concentrations of BTP appeared to be in steady state with a nonsignificant change during the interdialytic period (0.09 mg/l/day; $P = 0.71$). BTP equations had good performance in the validation cohort and are unlikely to be subject to same caveats as urea + creatinine equations. We found that sex coefficients were significant in our development models, suggesting that the association between GFR and BTP differs by sex. BTP can also decrease with corticosteroids and its use may not be reliable in patients receiving these

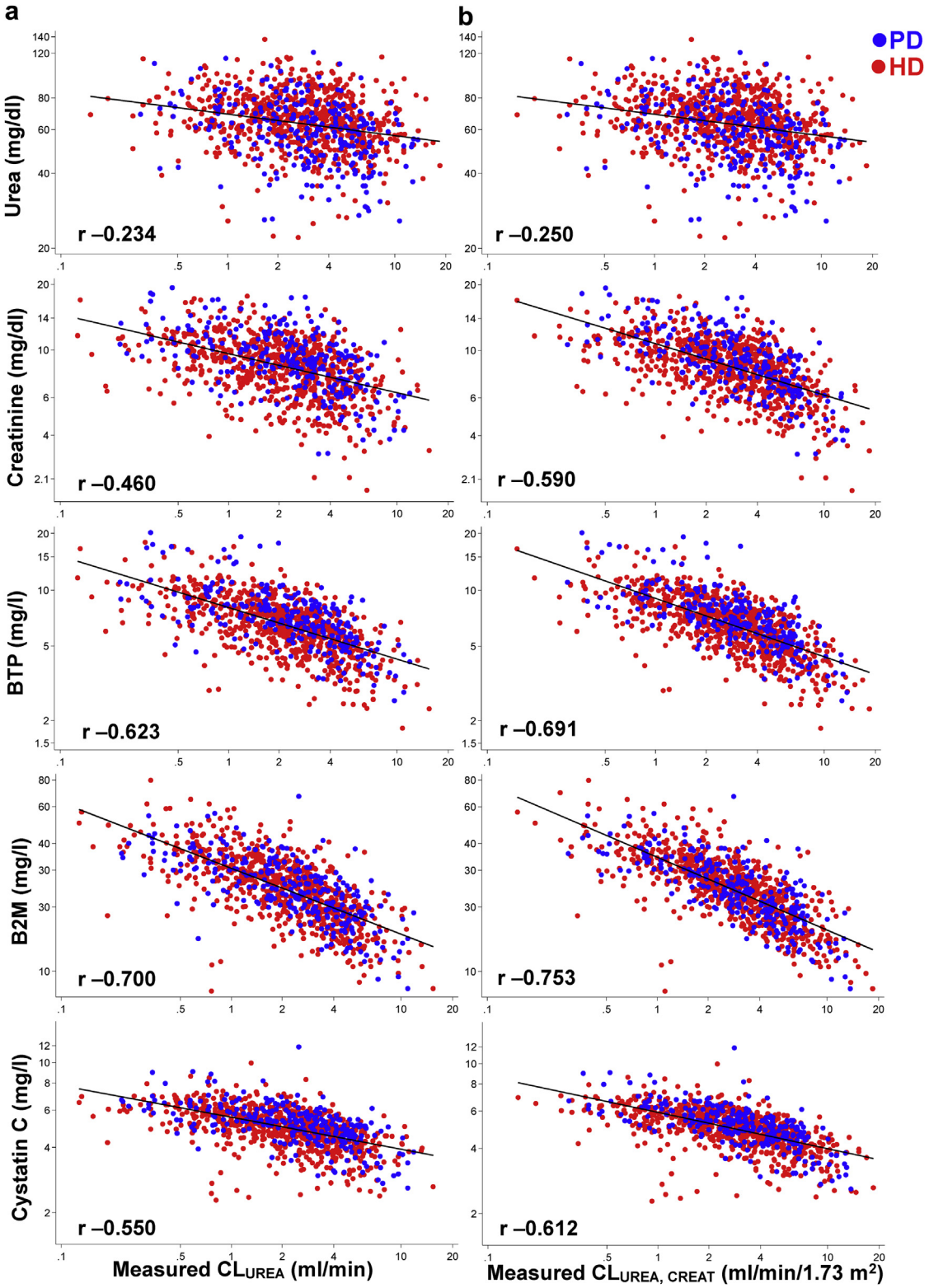


Figure 1 | Association between measured urinary clearances and endogenous filtration markers in 826 dialysis patients of the validation cohort, the NECOSAD. Scatterplots of natural log-transformed urea, creatinine, β-trace protein (BTP), β2-microglobulin (B2M), and cystatin C on the vertical (*y*) axis and measured urinary clearances on the horizontal (*x*) axis. Data from patients on peritoneal dialysis are displayed as blue dots, and data from patients on hemodialysis are displayed as red dots. Black line is the linear fit. Pearson correlation coefficients are displayed in the bottom left corner of each scatterplot. (**a**) Measured urinary urea clearance (CL_{UREA}) in ml/min. (**b**) Measured average of urinary urea and creatinine clearance ($CL_{UREA, CREAT}$) in ml/min/1.73 m^2. HD, hemodialysis; NECOSAD, Netherlands Cooperative Study on the Adequacy of Dialysis; PD, peritoneal dialysis.

Table 2 | RKF Study equations for estimating CL_{UREA} or $CL_{UREA,\ CREAT}$

CL_{UREA}, ml/min	
Urea, creatinine	CL_{UREA} (ml/min) = 1.1 × $UN^{0.949}$ × $creatinine^{-1.544}$
BTP	CL_{UREA} (ml/min) = 69 × $BTP^{-2.114}$ × 1.677 if male
B2M	CL_{UREA} (ml/min) = 1711 × $B2M^{-2.328}$ × 1.610 if male
Cystatin C	CL_{UREA} (ml/min) = 64 × cystatin $C^{-2.211}$
BTP, B2M	CL_{UREA} (ml/min) = 385 × $BTP^{-1.450}$ × $B2M^{-0.965}$ × 1.694 if male
$CL_{UREA,\ CREAT}$, ml/min/1.73 m^2	
Urea, creatinine	$CL_{UREA,\ CREAT}$ (ml/min/1.73 m^2) = 2.4 × $UN^{0.984}$ × $creatinine^{-1.868}$
BTP	$CL_{UREA,\ CREAT}$ (ml/min/1.73 m^2) = 95 × $BTP^{-2.16}$ × 1.652 if male
B2M	$CL_{UREA,\ CREAT}$ (ml/min/1.73 m^2) = 2852 × $B2M^{-2.417}$ × 1.592 if male
Cystatin C	$CL_{UREA,\ CREAT}$ (ml/min/1.73 m^2) = 123 × cystatin $C^{-2.468}$
BTP, B2M	$CL_{UREA,\ CREAT}$ (ml/min/1.73 m^2) = 673 × $BTP^{-1.406}$ × $B2M^{-1.096}$ × 1.670 if male

B2M, β2 microglobulin; BTP, β-trace protein; CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA,\ CREAT}$, average of urinary urea and creatinine clearance (ml/min/1.73 m^2); RKF, Residual Kidney Function; UN, serum urea nitrogen.
Coefficients for urea nitrogen and creatinine are expressed for concentrations in mg/dl. Conversion factors for units: creatinine in mg/dl to μmol/l, × 88.4; urea nitrogen in mg/dl to mmol/l, × 0.357.

medications.[29,30] B2M is a 11,600 Da protein that is a component of the major histocompatibility molecules present on all nucleated cells.[15] B2M is removed by high-flux hemodialysis and its concentrations increase in patients with malignancy and inflammation.[20] In our development cohort, there was a small interdialytic rise in B2M (1.27 mg/l/day; $P = 0.05$), which may affect interpretation of results after the long interdialytic interval or in patients with varying dialysis dose. Cystatin C is a 13,300 Da nonglycosylated protein that is expressed in all nucleated cells.[14] Cystatin C is also removed partially by high-flux hemodialysis; its concentrations can be affected by corticosteroid use, but probably not by inflammation.[21,29,30,31]In our development cohort, there was a significant interdialytic rise in cystatin C (0.30 mg/l/day; $P = 0.005$). Based on these findings, serum BTP equations may be the most reliable for assessing RKF in dialysis patients. However, while B2M and cystatin C assays are available for clinical use in the United States, BTP assay is not yet commercially available in the United States but was recently launched in Europe. Further studies are needed to carefully characterize the kinetics of these low molecular weight proteins in diverse dialysis populations and to validate our findings.

In previous studies, cystatin C is reported to be correlated with $CL_{UREA,\ CREAT}$ in dialysis patients, and a cystatin C equation to estimate $CL_{UREA,\ CREAT}$ was developed in a subset of the NECOSAD.[32] Other equations using BTP and cystatin C, including the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine and cystatin C equations, performed poorly for estimating $CL_{UREA,\ CREAT}$ in our study. A number of reasons may underlie this poor performance. First, because GFR estimating equations in nondialysis patients were developed in patients with higher GFR than in our study, the equation performance is not optimized at low GFR. Second, the coefficients for the serum markers in nondialysis studies reflect the influence of only endogenous non-GFR determinants and not the dialysis determinants. It is also possible that these non-GFR determinants change as GFR declines. Third, we did not standardize cystatin C measurement to International Federation of Clinical Chemistry standards. As a result, differences in performance of cystatin C RKF Study versus CKD-EPI equations may reflect differences in assay calibration. Similarly, variability in cystatin C assay over time could also partially account for differences in performance. There are no published equations for estimating CL_{UREA} in dialysis patients.

The performance of RKF Study urea + creatinine equation was significantly better than the CKD-EPI creatinine equation. This improved performance may reflect optimization of urea and creatinine coefficients in RKF Study equation to reflect nonrenal (dialytic) clearance. However, urea (60 Da) and creatinine (113 Da) are not in steady state between dialysis treatments. In the RKF Study, the interdialytic rise in serum urea nitrogen and creatinine was 10.8 and 1.3 mg/dl/day, respectively, while the patients were receiving a standardized diet. The rate of rise may be significantly different outside of this controlled environment and will affect the use of urea + creatinine equations in clinical practice. Nevertheless, because urea and creatinine are measured routinely in dialysis patients, the RKF Study urea + creatinine equation might be used as a screening tool to estimate RKF in patients with self-reported urine output ≥1 cup/day, without additional cost. Low molecular weight proteins, and in particular BTP, may then be used for more reliable RKF estimation and clinical decision making.

RKF is strongly associated with improved survival in hemodialysis and peritoneal dialysis patients; each 1 ml/min/1.73 m^2 higher $CL_{UREA,\ CREAT}$ is associated with an 11% to 48% lower risk of death.[1–4,20] Besides excretion of freely filtered solutes (e.g., urea, creatinine), RKF also enables excretion of protein-bound solutes (e.g., *p*-cresol sulfate, indoxyl sulfate), which are cleared by tubular secretion but not effectively removed by dialysis.[9] Continuous volume excretion reduces volume overload and left ventricular hypertrophy.[6,8] Preserving RKF may improve survival in dialysis patients, but the cumbersome nature of urine collections has greatly impeded advances in this area. RKF is also a strong confounder in dialysis studies and incomplete adjustment for RKF can lead to biased results. Our results now present a new opportunity to assess RKF without urine collections and can potentially overcome challenges to incorporating RKF in dialysis care and research.

In routine clinical practice, hemodialysis adequacy is assessed by equation-estimated dialyzer urea clearance for 1 dialysis session ($spKt/V_{UREA}$; target ≥1.4),[7] which can be used to calculate a cumulative weekly standard Kt/V_{UREA} ($stdKt/V_{UREA}$; target ≥2.3).[7] Calculation of $stdKt/V_{UREA}$ allows comparison of dose across different hemodialysis regimens

Table 3 | RKF Study CL_{UREA} and $CL_{UREA,\ CREAT}$ estimating equations' performance in 826 patients from the validation cohort (NECOSAD)

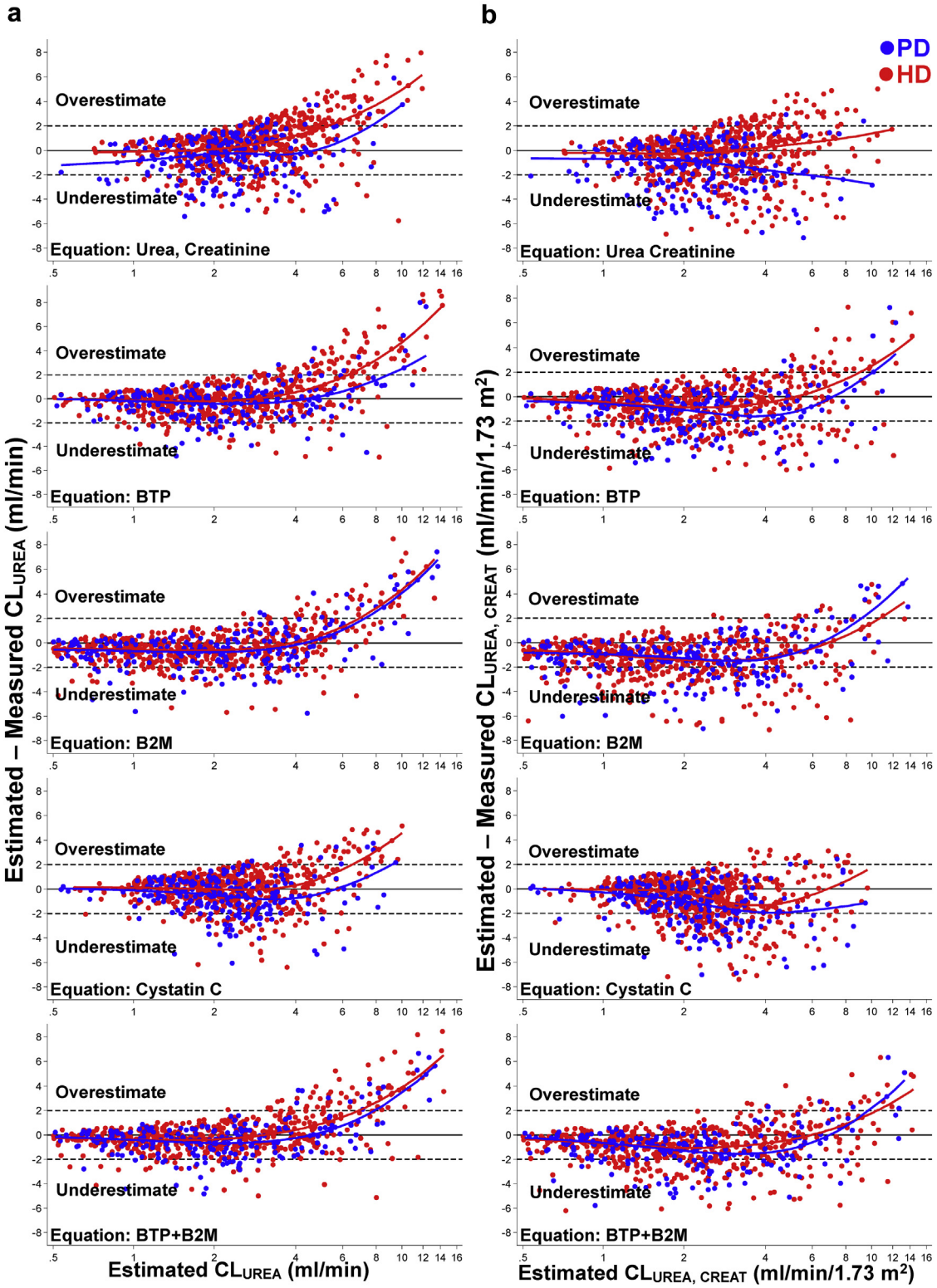
RKF Study equation	Markers	Other variables	RMSE[a]	Median bias (95%CI)[b,c] Measured clearance – Estimated clearance	Precision (95% CI)[d,e] IQR of bias	Accuracy (95% CI)[f,g] Estimates within ±2 ml/min of CL_{UREA}
CL_{UREA}, ml/min				ml/min	ml/min	%
	Urea, creatinine		0.753	0.2 (0.01, 0.3)	2.2 (2.1, 2.4)	75 (72, 78)
	BTP	Sex	0.612	0.4 (0.3, 0.5)[h]	1.8 (1.6, 2.0)[i]	81 (78, 83)[i,j]
	B2M	Sex	0.584	0.7 (0.6, 0.8)[h]	1.6 (1.5, 1.7)[i]	79 (76, 81)[i,k]
	Cystatin C		0.667	0.5 (0.4, 0.6)[h]	2.0 (1.8, 2.1)	79 (76, 82)[i,j]
	BTP, B2M	Sex	0.569	0.5 (0.4, 0.6)[h]	1.5 (1.4, 1.7)[i]	81 (79, 84) [i,j]
$CL_{UREA,\ CREAT}$, ml/min/1.73 m^2				ml/min/1.73 m^2	ml/min/1.73 m^2	Estimates within ±2 ml/min/1.73 m^2 of $CL_{UREA,\ CREAT}$, %
	Urea, creatinine		0.669	0.3 (0.2, 0.5)[h]	2.7 (2.5, 2.9)	68 (65, 72)
	BTP	Sex	0.556	0.6 (0.5, 0.7)[h]	2.1 (2.0, 2.3)	71 (68, 74)
	B2M	Sex	0.553	1.0 (0.9, 1.1)[h]	1.9 (1.7, 2.1)	69 (66, 72)
	Cystatin C		0.605	0.7 (0.5, 0.9)[h]	2.3 (2.1, 2.5)	72 (69, 75)[i,k]
	BTP, B2M	Sex	0.506	0.7 (0.6, 0.8)[h]	1.8 (1.6, 1.9)	75 (72, 78)[i,j]

B2M, β2 microglobulin; BTP, β-trace protein; CI, confidence interval; CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA,\ CREAT}$, average of urinary urea and creatinine clearance (ml/min/1.73 m^2); IQR, interquartile range; NECOSAD, The Netherlands Cooperative Study on the Adequacy of Dialysis; RKF, Residual Kidney Function; RMSE, root-mean-square error.
In the NECOSAD Study, 826 participants had 989 clearance measurements. Performance data are only for the first measurement ($n = 826$).
[a]RMSE from linear regression of natural log-transformed measured urinary clearance on natural log-transformed estimated urinary clearance. A smaller RMSE implies a better model fit.
[b]Bias is defined as the median difference between measured clearance and estimated clearance. Confidence intervals are calculated by bootstrapping with 2000 replicates.
[c]Significance of bias in estimation using BTP, B2M, and cystatin C equations compared with urea + creatinine equation was determined by Wilcoxon matched-pairs signed-ranks test). All P values are <0.001.
[d]Precision is defined as IQR of the median bias. CI are calculated by bootstrapping with 2000 replicates.
[e]Significance of the precision of estimation using BTP, B2M, and cystatin C equations compared with urea + creatinine equation was determined by IQR regression with bootstrapped (2000 replicates) standard errors. All significant differences have P values <0.001.
[f]Accuracy is defined as estimates within ±2 ml/min of CL_{UREA} or ±2.5 ml/min/1.73 m^2 of $CL_{UREA,\ CREAT}$. CI are calculated by bootstrapping with 2000 replicates.
[g]Significance of the accuracy of estimation using BTP, B2M, and cystatin C equations compared with urea + creatinine equation was determined by McNemar chi-square test.
[h]Worse result than the urea + creatinine equation.
[i]Better result than the urea + creatinine equation.
[j]$P < 0.001$.
[k]$P < 0.05$.

such as 3 times a week in-center hemodialysis and 5 to 7 times a week home/frequent hemodialysis.[33] Peritoneal dialysis adequacy is assessed by quarterly peritoneal urea clearance measurement and expressed as weekly stdKt/V_{UREA} (target ≥1.7).[11] CL_{UREA} from a timed-urine collection is routinely incorporated into peritoneal dialysis prescription. Equations to incorporate CL_{UREA} into the stdKt/V_{UREA} calculations for hemodialysis patients are also available.[33] The 2015 National Kidney Foundation KDOQI Guidelines for Hemodialysis Adequacy recommend that CL_{UREA} can be included in adequacy calculations provided it is measured periodically.[34] The KDOQI 2006 Peritoneal Dialysis guidelines recommend that CL_{UREA} can be incorporated in dialysis dose if urine volume is ≥200 ml per day.[11] The RKF Study equations will allow estimation of CL_{UREA} from serum markers without urine collection. The estimated CL_{UREA} can then be used to adjust dialysis dose by incorporating it in stdKt/V_{UREA}. This strategy can used for peritoneal dialysis patients to adjust dialysate volume or the number of fills. For home/frequent hemodialysis patients, lower dialysate volume can be used for patients treated with NxStage System One (Lawrence, MA), and for patients without volume overload, the frequency of treatments can be reduced to 3 to 4 times per week from 5 to 7 times per week. Similarly, in-center hemodialysis patients without volume overload that have substantial CL_{UREA} can be dialyzed less frequently or for shorter duration. Further studies are needed to validate the safety and effectiveness of these strategies.

The focus of our study was cross-sectional estimation of RKF. However, we recognize the clinical importance of repeated measurements over time in a single individual. In the subgroup of NECOSAD participants with repeated measurements ($n = 162$), measured clearances declined over time; CL_{UREA} by −0.7 ml/min (IQR: −1.4, −0.01) and $CL_{UREA,\ CREAT}$ by −1.1 ml/min/1.73 m^2 (IQR: −1.9, −0.05). The decline in measured clearance correlated with increased concentrations of endogenous filtration makers, particularly BTP, B2M, and cystatin C (Figure S2). Equation-estimated clearance also declined over time (Table S9). Although the first and repeat equation-estimated clearances were moderately highly correlated, the equations underestimated the change in clearance over time. For example, the median change in equation-estimated CL_{UREA} with BTP and B2M equations was −0.2 ml/min and −0.4 ml/min, respectively, compared with change in measured CL_{UREA} of −0.7 ml/min. These findings highlight the need for improving performance of estimation equations and should be kept in mind while monitoring individual patients.

Strengths of our study include careful urine collections in the development cohort (RKF Study) under near ideal conditions, which allowed reliable measurements of CL_{UREA} and $CL_{UREA,\ CREAT}$; a highly rigorous prespecified analytic plan for equation development; use of multiple endogenous filtration markers; and a large external validation cohort of hemodialysis and peritoneal dialysis patients in a different country with a different racial-ethnic composition and body weight than the validation cohort, which greatly improves the generalizability of our results. Limitations include few



patients treated with peritoneal dialysis in the development cohort and urine collections at home in NECOSAD, which may have introduced measurement error and reduced the equations' performance. In the development cohort, we did not perform bladder ultrasound to check for bladder emptying, which could contribute to underestimation of urinary clearance. GFR can vary during the interdialytic interval in dialysis patients.[35] We did not standardize the day of the week for clearance measurements, which could contribute to underestimation of CL_{UREA}. The relatively large root-mean-square error suggests presence of high relative variability (log scale measures variation as a fraction of the absolute value of the gold standard) that is not completely captured by the variables in the estimating equations, highlighting the need to improve estimates for individual prediction and clinical decision making. However, we must also recognize that the gold standard itself has an error margin, which limits how well it can be predicted.[36] As we did not standardize cystatin C measurements, the internal comparisons remain valid but differences in performance with external equations and future studies may also be due to laboratory measurement error. However, impact on estimation is likely to be minimal when measured in units of ml/min/1.73 m^2. We did not exclude patients with thyroid disease or steroid use in the development cohort and this may also affect the performance of cystatin C equation.

In conclusion, we have developed equations to estimate CL_{UREA} and $CL_{UREA,\ CREAT}$ in dialysis patients without requiring urine collections (Table 2). These equations have good performance and diagnostic accuracy. In particular, serum BTP appears to be in steady state during the interdialytic interval and BTP equations may not be influenced by diet and dialysis schedules compared with equations using other filtration markers. These RKF estimation equations are valid for patients with self-reported urine output ≥1 cup/day that are treated with peritoneal dialysis or conventional (nonconvective) hemodialysis. Further research is needed to determine whether dialysis dose can be safely modified using estimating equations instead of timed-urine collections. A Web calculator is available at http://www.kidneymodels.org/rkf.

MATERIALS AND METHODS

Study design and data collection

We developed the equations in the RKF Study, a prospective cohort of dialysis patients in Baltimore, Maryland.[37] From November 2011 to October 2014, we recruited dialysis patients from 8 outpatient dialysis units. Inclusion criteria were age ≥18 years, English speaker, and self-reported ability to produce ≥1 cup/day (approximately 250 ml) of urine. Exclusion criteria included prior kidney transplant. Patients underwent carefully supervised urine clearance measurements at a baseline visit ($n = 44$). Additionally, 9 patients underwent repeat measurements within 6 weeks of initial visit (median: 33 days; IQR: 30, 40) and 8 at 12 months (median: 371 days; IQR: 285, 385). We used data from these 61 clearance measurements (44 initial visits and 17 repeat visits), collected under near-ideal setting, for equation development.

We validated the equations in the NECOSAD, a large multicenter prospective cohort study of incident hemo- and peritoneal dialysis patients in the Netherlands that recruited patients from 38 dialysis centers from January 1997 to January 2005.[2,3] Inclusion criteria were age ≥18 years and starting renal replacement therapy for the first time. The present analysis includes 826 patients with stored specimens and available data on RKF.

The Johns Hopkins Medicine Institutional Review Board approved the study. The NECOSAD was approved by the Medical Ethics boards of all participating centers.

Renal clearance measurement

In both studies, RKF was assessed by a timed-urine collection to measure urinary solute clearances. In the RKF Study, we performed clearance measurements in hemodialysis patients on an interdialytic day, at least 12 hours or more after the last hemodialysis session (Tuesday or Thursday for patients dialyzing on a Monday, Wednesday, Friday schedule and Sunday, Wednesday, or Friday for patients on a Tuesday, Thursday, Saturday dialysis schedule). We performed the clearance measurements under carefully supervised conditions, during a 24-hour inpatient research visit in the Johns Hopkins Bayview Clinical Research Unit in Baltimore, Maryland. Prior to the visit, we verified that patients were on a stable dose of antihypertensive medications. We instructed the patients to eat a light meal on the evening before the visit and a light breakfast on the morning of the visit. During the visit, we served food from standardized menus with the following average composition: protein 64 ± 1 g/day; potassium 1.7 ± 0.1 g/day; sodium 1.7 ± 0.4 g/day; and phosphate 0.8 ± 0.5 g/day. We allowed daily fluid intake of 1000 ml/day and only allowed noncaffeinated drinks. During the visit, patients were allowed to ambulate in their room and an adjacent lounge. Patients were encouraged not to smoke but were allowed to smoke if they requested to do so. We collected blood samples at the start of measurement (0 minutes), at 2 hours, and at 24 hours when the urine collection ended. For the duration of the visit, trained nurses monitored and regularly reminded the patients to collect all voided urine. We calculated urinary CL_{UREA} and CL_{CREAT} from 24-hour urine collections as follows: urine concentration ×urine volume ÷ mean serum concentration (from measurements at 0, 2 hours, and 24 hours). We expressed

Figure 2 | Association between estimated and measured clearances in 826 dialysis patients of the validation cohort, the NECOSAD. The difference between estimated and measured clearance is presented on the vertical (*y*) axis and estimated clearance on the horizontal (*x*) axis. Positive numbers on the *y*-axis represent overestimation of measured clearance and negative numbers represent underestimation. Extreme observations, defined as estimated clearance (*x*-axis) >99th percentile or <0.5 ml/min for CL_{UREA} (0.5 ml/min/1.73 m^2 for $CL_{UREA,\ CREAT}$) and the difference between estimated and measured clearance (*y*-axis) >99th percentile or <1st percentile, are excluded. Data from patients on peritoneal dialysis are displayed as blue dots, and data from patients on hemodialysis are displayed as red dots. Blue and red lines are model fits from median quantile regression of bias on measured clearance modeled as restricted cubic spline with 4 quantile knots. Solid black line represents bias = 0. (**a**) Results for CL_{UREA} in ml/min. (**b**) Results for $CL_{UREA,\ CREAT}$ in ml/min/1.73 m^2. B2M, β2 microglobulin; BTP, β-trace protein; CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA,\ CREAT}$, average of urinary urea and creatinine clearance (ml/min/1.73 m^2); HD, hemodialysis; NECOSAD, The Netherlands Cooperative Study on the Adequacy of Dialysis; PD, peritoneal dialysis.

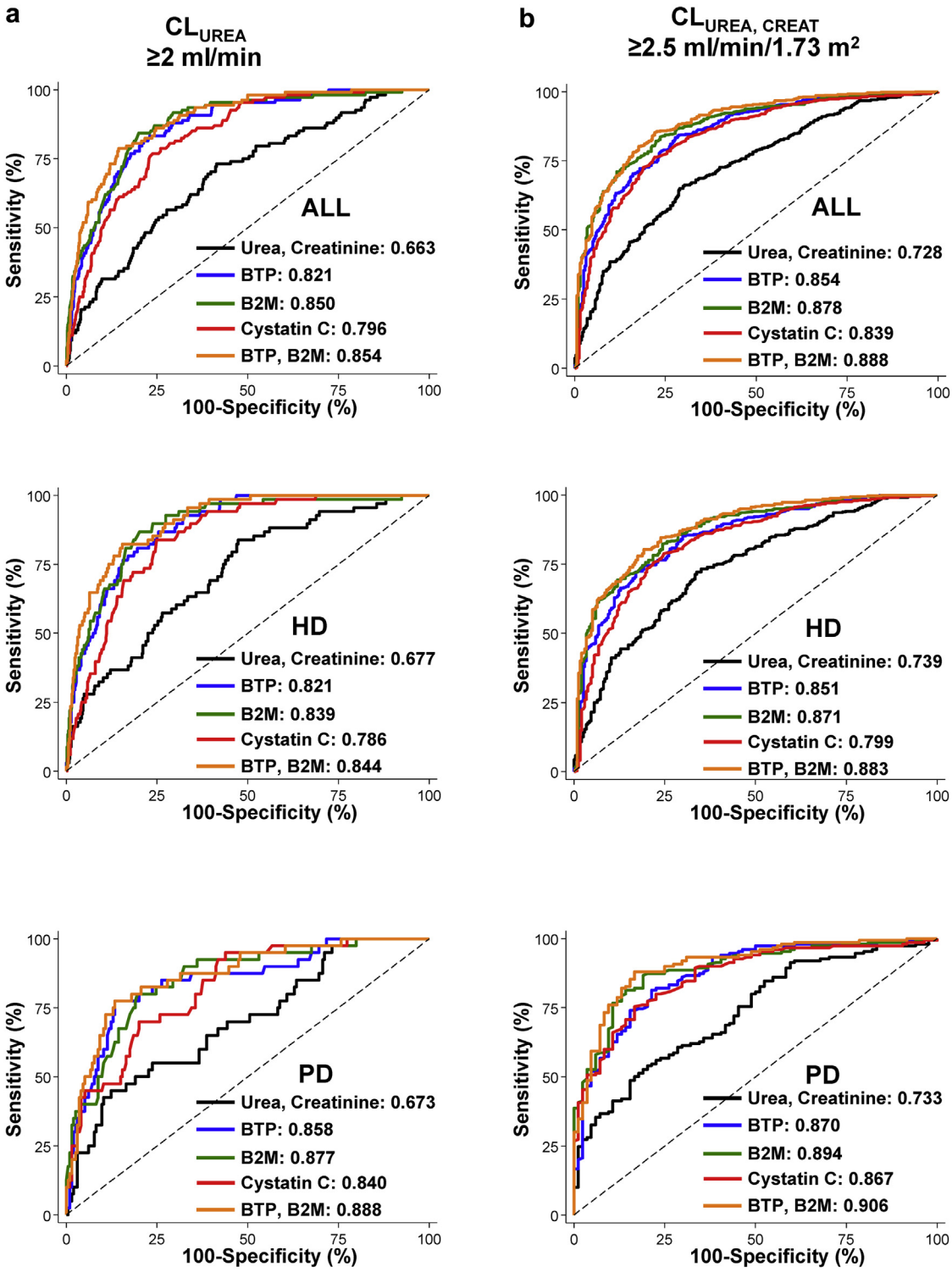


Figure 3 | ROC curves for the diagnostic accuracy of estimating equations in 826 dialysis patients of the validation cohort, the NECOSAD. Sensitivity (%) is presented on the *y*-axis and 100-Specificity (%) is presented on the *x*-axis. Solid black line was calculated using the urea and creatinine equation, solid blue line the BTP equation, solid green line the B2M equation, solid red line the cystatin C equation, and orange line the BTP + B2M equation. Results are presented overall and stratified by patients treated with hemodialysis or peritoneal dialysis. Area under the receiver operating characteristic (ROC) curve is presented as numbers in each panel. (**a**) Diagnostic accuracy for estimating CL_{UREA} ≥2 ml/min. (**b**) Diagnostic accuracy for estimating $CL_{UREA, CREAT}$ ≥2.5 ml/min/1.73 m^2. B2M, β2 microglobulin; BTP, β-trace protein; CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA, CREAT}$, average of urinary urea and creatinine clearance (ml/min/1.73 m^2); HD, hemodialysis; NECOSAD, Netherlands Cooperative Study on the Adequacy of Dialysis; PD, peritoneal dialysis.

CL_{UREA} in ml/min to allow incorporation in Kt/V_{UREA}, which uses urea volume of distribution rather than body surface area. We also calculated the average of urinary urea and creatinine clearance ($CL_{UREA,\ CREAT}$) and expressed it per 1.73 m^2 of body surface area calculated by Dubois formula[38] to allow comparability to GFR estimating equations in nondialysis patients. We used CL_{UREA} as the reference test for estimating urea clearance and $CL_{UREA,\ CREAT}$ as the reference test for estimating GFR.

In the peritoneal dialysis patients of the NECOSAD, we used timed 24-hour urine collections directly prior to a monitoring visit to the outpatient clinic, where a blood sample was taken. We used this sample to calculate urinary clearances. The hemodialysis patients collected all urine produced during the entire interdialytic interval and blood samples were drawn at the end of the preceding hemodialysis session and directly before the next hemodialysis. We used the mean of these 2 values for the urinary clearance calculations in hemodialysis patients.[39] We analyzed data from samples obtained at 3 or 12 months after dialysis initiation in NECOSAD.

Laboratory methods

We performed all laboratory measurements at the University of Minnesota's Advanced Research and Diagnostic Laboratory. Serum and urine urea and creatinine were measured on a Roche COBAS 6000 Analyzer (Roche Diagnostics, Indianapolis, IN), and BTP, B2M, and cystatin C were measured on a Siemens ProSpec Nephelometer (Siemens Medical Solutions, Malvern, PA). Assay precision, characteristics, and normal ranges are described in Table S10. In NECOSAD, creatinine (mainly using the alkaline picrate method) and urea had previously been measured at the local laboratories. Earlier analyses in NECOSAD had shown that the method of creatinine measurement had a negligible effect on creatinine concentrations in the presence of very high serum concentrations in dialysis patients.

Analyses in the development dataset—RKF Study

We developed separate equations to estimate CL_{UREA} (in ml/min) and $CL_{UREA,\ CREAT}$ (in ml/min/1.73 m^2) based on serum urea and creatinine (together) and BTP, B2M, and cystatin C alone and in combination with each other. We used serum markers from the 24-hour time point (predialysis values) as predictors in model development as the blood samples from this time point can be readily obtained in clinical practice. We prespecified a process for equation development similar to our methods for estimating GFR in nondialysis patients.[14,40–42] We transformed continuous variables to natural logarithms to stabilize variance. We compared the correlation between log-transformed markers and log-transformed CL_{UREA} (or $CL_{UREA,\ CREAT}$). We used least square linear regression to relate log-transformed measured CL_{UREA} (or $CL_{UREA,\ CREAT}$) to log serum markers assessing linearity from lowess smoothed plots (bandwidth = 0.8). We then considered inclusion of age, sex, and race in the models, defining the significance threshold for model entry as $P < 0.1$ and for including interactions as $P < 0.01$. We retained statistically significant variables in the model if they reduced the root-mean-square error by ≥2%. Root-mean-square error measures the typical deviation of individual observations from the model prediction providing precision with which the dependent variable (CL_{UREA} or $CL_{UREA,\ CREAT}$) can be predicted. A smaller root-mean-square error implies a better model fit. In sensitivity analyses, we forced age, sex, and race into the estimating equations models and assessed whether they improved equation performance. For equation building, we used least square linear regression on data from 61 visits for 44 participants with the cluster option in STATA (StataCorp, College Station, TX) calculating robust standard errors after allowing for within individual correlations. To assess the change in markers over time, we used a random effects model with a population-averaged estimator.

Analyses in the external validation dataset—NECOSAD

We compared the baseline characteristics of RKF Study participants with the NECOSAD participants, overall and by dialysis modality, using *t*-test for continuous variables and chi-square test for categorical variables. We compared the performance of the RKF Study CL_{UREA} and $CL_{UREA,\ CREAT}$ estimating equations overall and in subgroups of patients receiving peritoneal dialysis or hemodialysis. We excluded repeat measurements over time while assessing performance. We also compared the performance of the RKF Study $CL_{UREA,\ CREAT}$ equations with other published GFR estimating equations.[14,27,28,32,40,41] There are no published equations for estimating CL_{UREA}. In the subset of patients with repeat measurements, we compared the correlations between repeat measurements and repeat estimations (excluding 1 patient with increase in CL_{UREA} >9 ml/min on repeat measurement). We only tested RKF Study equations' performance in NECOSAD and did not change RKF Study equations based on the NECOSAD data.

Metrics for equation performance

We compared, measured, and estimated CL_{UREA} (or $CL_{UREA,\ CREAT}$) graphically by plotting the difference (measured CL_{UREA} – estimated CL_{UREA}) against estimated CL_{UREA} as the estimates are the metric observed in clinical practice (residual vs. fitted values plot). We defined bias as the median difference and precision as the IQR of this difference. We defined accuracy as estimates within ±2 ml/min of measured CL_{UREA} (or ±2 ml/min/1.73 m^2 of measured $CL_{UREA,\ CREAT}$). We choose this absolute difference of 2 ml/min rather than a relative percentage change as this threshold is clinically relevant (used as a cutoff CL_{UREA} for dialysis adequacy consideration by the 2006 KDOQI Hemodialysis Adequacy Guidelines)[7] and because at a low level of kidney function, small absolute differences in clearance can result in a large relative difference. We calculated CI for the metrics using bootstrapping with 2000 replicates. We determined the significance of differences between equations using the Wilcoxon matched-pairs signed-ranks test for bias, IQR regression for precision, and McNemar test for accuracy. We assessed differences in equation performance between dialysis modalities using median quantile regression for bias, IQR regression for precision, and 2-sample test of proportions for accuracy. We also assessed the sensitivity, specificity, positive and negative predictive values, and area under the receiver operating characteristic curve of the equations for estimating measured CL_{UREA} ≥2 ml/min or $CL_{UREA,\ CREAT}$ ≥2.5 ml/min/1.73 m^2, which is the mean $CL_{UREA,\ CREAT}$ in the development data when CL_{UREA} is 2 ml/min.

We performed all analyses using STATA (version 13.1).

DISCLOSURE

TS is supported by grant no. K23-DK-083514 from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health and has received speaker fees from Siemens. ASL has received funding paid to Tufts Medical Center for research and contracts with the National Institutes of Health, National Kidney Foundation, Amgen, Pharmalink AB, Gilead Sciences; and has a provisional patent filed 15 August 2014—Precise estimation of glomerular filtration rate from multiple biomarkers (licensing under negotiation). LAI has received funding paid to Tufts Medical Center for research and contracts with the National Institutes of Health, National Kidney Foundation, Pharmalink AB, and Gilead Sciences;

has a consulting agreement with Otsuka; and has a provisional patent filed 15 August 2014—Precise estimation of glomerular filtration rate from multiple biomarkers (licensing under negotiation). JHE is a consultant for Gentian, which is a Norwegian manufacturer of reagents for clinical cystatin C measurement procedures, and his research laboratory has received free or steeply discounted reagents from Siemens for measurement of β-trace protein, cystatin C, and β-2 microglobulin. JC has a provisional patent filed 15 August 2014—Precise estimation of glomerular filtration rate from multiple biomarkers (licensing under negotiation). All the other authors declared no competing interests.

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The reagents for β-trace protein, β2-microglobluin and cystatin C assays were provided by Siemens to the University of Minnesota, where the measurements were performed. Siemens had no role in the design, analysis, and interpretation of data or the preparation of this manuscript.

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Study data were collected and managed using REDCap electronic data capture tools hosted at Johns Hopkins University.[43] REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing (i) an intuitive interface for validated data entry; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for seamless data downloads to common statistical packages; and (iv) procedures for importing data from external sources.

Parts of this work were presented at the 2014 Annual Meeting of the American Society of Nephrology in Philadelphia, PA, 12 to 16 November 2014.

SUPPLEMENTARY MATERIAL

Figure S1. Association between measured urinary clearances and endogenous filtration markers in 44 dialysis patients in development cohort, the Residual Kidney Function Study. Scatterplots of natural log-transformed urea, creatinine, β-trace protein (BTP), β2-microglobulin (B2M), and cystatin C on the vertical (*y*) axis and measured urinary clearances on the horizontal (*x*) axis. Data from patients on peritoneal dialysis are displayed as blue dots and data from patients on hemodialysis are displayed as red dots. Black line is the linear fit. Pearson correlation coefficients are displayed in the bottom left corner of each scatterplot. (**a**) Measured urinary urea clearance (CL_{UREA}) in ml/min. (**b**) Measured average of urinary urea and creatinine clearance ($CL_{UREA,\ CREAT}$) in ml/min/1.73 m^2. CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA,\ CREAT}$, average of urinary urea and creatinine clearance (in ml/min/1.73 m^2); HD, hemodialysis; PD, peritoneal dialysis.

Figure S2. Association between change in measured urinary clearances and endogenous filtration markers in 162 dialysis patients in validation cohort, the NECOSAD (Netherlands Cooperative Study on the Adequacy of Dialysis). Scatterplots of change in urea, creatinine, β-trace protein, β2-microglobulin, and cystatin C on the vertical (*y*) axis and change in measured urinary clearances on the horizontal (*x*) axis. Data from patients on peritoneal dialysis are displayed as blue dots and data from patients on hemodialysis are displayed as red dots. Black line is the linear fit. Pearson correlation coefficients are displayed in the bottom left corner of each scatterplot. (**a**) Measured urinary urea clearance (CL_{UREA}) in ml/min. (**b**) Measured average of urinary urea and creatinine clearance ($CL_{UREA,\ CREAT}$) in ml/min/1.73 m^2. CL_{UREA}, urinary urea clearance (ml/min); $CL_{UREA,\ CREAT}$, average of urinary urea and creatinine clearance (in ml/min/1.73 m^2); HD, hemodialysis; PD, peritoneal dialysis.

Table S1. Correlation among CL_{UREA}, $CL_{UREA,\ CREAT}$, and endogenous filtration markers

Table S2. Association of CRP with endogenous filtration markers in the NECOSAD study

Table S3. Coefficients for CL_{UREA} and $CL_{UREA,\ CREAT}$ estimating equations in the development data (Residual Kidney Function Study)

Table S4. Residual kidney function estimating equations performance in the development data (Residual Kidney Function Study)

Table S5. Comparison of the performance of Residual Kidney Function Study CL_{UREA} and $CL_{UREA,\ CREAT}$ estimating equations' between 587 hemodialysis and 239 peritoneal dialysis patients from the external validation cohort (NECOSAD)

Table S6. Diagnostic test performance of Residual Kidney Function Study CL_{UREA} and $CL_{UREA,\ CREAT}$ estimating equations' overall and stratified by dialysis modality in the external validation cohort (NECOSAD)

Table S7. Other $CL_{UREA,\ CREAT}$ estimating equations' performance in 826 patients from the validation cohort (NECOSAD)

Table S8. Correlation of change in filtration markers with measured urinary clearances over time in 162 patients of the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) with repeat measurements

Table S9. Assessing change over time with RKF Study estimating equations in 162 patients of the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) with repeat measurements

Table S10. Serum and urinary analytes measurement

Supplementary material is linked to the online version of this paper at www.kidney-international.org.

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