Predicting residual kidney function in hemodialysis patients using serum β-trace protein and β2-microglobulin

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Residual kidney function (RKF) contributes significant solute clearance in hemodialysis patients. Kidney Diseases Outcomes Quality Initiative (KDOQI) guidelines suggest that hemodialysis dose can be safely reduced in those with residual urea clearance (KRU) of 2 ml/min/1.73 m^2 or more. However, serial measurement of RKF is cumbersome and requires regular interdialytic urine collections. Simpler methods for assessing RKF are needed. β-trace protein (βTP) and β2-microglobulin (β2M) have been proposed as alternative markers of RKF. We derived predictive equations to estimate glomerular filtration rate (GFR) and KRU based on serum βTP and β2M from 191 hemodialysis patients based on standard measurements of KRU and GFR (mean of urea and creatinine clearances) using interdialytic urine collections. These modeled equations were tested in a separate validation cohort of 40 patients. A prediction equation for GFR that includes both βTP and β2M provided a better estimate than either alone and contained the terms 1/βTP, 1/β2M, 1/serum creatinine, and a factor for gender. The equation for KRU contained the terms 1/βTP, 1/β2M, and a factor for ethnicity. Mean bias between predicted and measured GFR was 0.63 ml/min and 0.50 ml/min for KRU. There was substantial agreement between predicted and measured KRU at a cut-off level of 2 ml/min/1.73 m^2. Thus, equations involving βTP and β2M provide reasonable estimates of RKF and could potentially be used to identify those with KRU of 2 ml/min/1.73 m^2 or more to follow the KDOQI incremental hemodialysis algorithm.

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Residual kidney function (RKF) is of significant prognostic importance to patients on hemodialysis (HD).[1] It has many clinical advantages including improved nutrition,[2] anemia, and phosphate control.[3] Even small amounts of RKF can provide significant benefit.

The Kidney Diseases Outcomes Quality Initiative (KDOQI) suggests that minimum dialysis Kt/V targets may be reduced in those with residual urea clearance (KRU) ≥2 ml/min/1.73 m^2. The European Best Practice Guidelines (EBPG) recommend measuring RKF in HD patients using the mean of urea and creatinine clearances and offer suggestions to incorporate this into the HD prescription to allow individual adjustment of dialysis prescription to meet minimum dialysis adequacy targets.[4,5] However, measurement of urea and creatinine clearances requires an interdialytic urine collection,[6] which can be difficult and inconvenient for patients because RKF has to be monitored at least every 1 to 3 months for incremental HD to be practiced safely.[7] Serum biomarkers that obviate the need for regular urine collections would be desirable.

Urea and creatinine are imperfect biomarkers of kidney function because of external influences by factors such as muscle mass, gender, diet, and nutritional status. Hence there has been interest in novel alternative serum biomarkers, especially cystatin C, β-trace protein (βTP), and β2-microglobulin (β2M).[8–11] Use of cystatin C in dialysis patients is limited because nonrenal clearance of cystatin C is significant and greatly exceeds its renal clearance in this setting.[12,13] βTP is a 23 kDa glycoprotein, also known as lipocalin type prostaglandin D synthase, and is expressed in a number of organs including the brain, retina, testes, heart, and kidney.[14] It is virtually exclusively excreted by the kidneys,[15] and serum levels of βTP concentration correlate well with residual urine volumes in HD patients,[16] though its ability to predict RKF in the HD setting has not been explored. β2-microglobulin (β2M) has a molecular weight of 11.8 kDa and accumulates in kidney failure. β2M levels have a close relationship with RKF in HD[17] and peritoneal dialysis.[18,19] RKF is the most significant determinant of β2M levels in HD patients and has a greater influence on these levels than the convective clearance provided by hemodiafiltration (HDF).[17,20]

Hence βTP and β2M are promising candidates as predictors of RKF in the HD setting. Both have limitations

though; β2M levels may increase with conditions such as lupus and malignancy,[21,22] and the clinical factors that influence βTP are not well understood, although factors such as gender,[9] ethnicity,[23] atherosclerosis,[24] and inflammation[9] have been implicated.

The aim of this study was to evaluate the usefulness of βTP and β2M as estimates of RKF in HD patients. Clinical determinants of βTP and β2M in the HD setting were explored, and prediction equations to estimate residual urea clearance (KRU) and glomerular filtration rate (GFR) in HD patients were constructed based on serum levels of βTP and β2M. The predictive equations were compared with KRU and GFR measured using interdialytic urea and creatinine clearances in a separate validation cohort of HD patients. We also explored the ability of predictive equations to identify HD patients with KRU ≥2 ml/min/1.73 m^2 to follow the KDOQI incremental HD algorithm.

RESULTS

Characteristics of the study cohort

The study cohort consisted of 231 prevalent HD patients based at the East & North Herts NHS Trust; 191 patients were randomly selected into a modeling group for derivation of equations for predicting parameters of RKF based on serum levels of βTP and β2M, and the remaining 40 patients were used for validation of the final constructed equations (Table 1). There were no significant differences between the modeling and validation cohorts in terms of age, anthropometric parameters, ethnicity, blood pressure, dialysis adequacy, diabetes prevalence, primary renal disease, and Charlson co-morbidity index. Serum βTP and β2M concentrations were similar in both groups. The modeling cohort had a higher GFR than the validation cohort (1.72 vs. 0.74 ml/min/1.73 m^2), whereas the validation cohort had a higher prevalence of malignant disease and a higher median C-reactive protein level.

Clinical determinants of βTP and β2M

Clinical determinants of serum βTP and β2M levels in HD were sought using univariable and multivariable regression analysis of clinical and demographic data from the modeling cohort. Independent predictors of βTP and β2M are shown in Table 2 and Table 3, respectively.

Predictors of βTP

In multivariable analysis, significant positive associations with βTP were found for male gender and the prevalence of atheromatous disease (Table 2). There were inverse associations for age, body surface area, GFR, and treatment with HDF. Caucasian ethnicity, prevalence of malignant disease, ultrafiltration volume, dialyzer Kt/V, diuretic use, and mean interdialytic weight gain were associated with βTP in univariable analysis only.

Predictors of β2M

In multivariable analysis, significant associations with β2M were found with GFR and diabetic status (Table 3). Weight, male gender, dialysis vintage, ultrafiltration volume, mean interdialytic weight gain, dialyzer Kt/V, and diuretic use were associated in univariable analysis only. No significant associations were found with C-reactive protein, HDF treatment, or convective volume.

Development of prediction equations for KRU and GFR using βTP and β2M

Linear regression models for KRU and GFR were determined using the modeling cohort in three phases: (i) using βTP alone, (ii) using β2M alone, and (iii) using both βTP and β2M. Other relevant covariates were used in each case. The best constructed models are shown in Table 4. Integrated Discrimination Improvement analysis[25] was used to assess the predictive accuracy of the equation that incorporated both βTP and β2M (model 3) compared with the best model using a single biomarker (model 1 or 2) for cut-off levels 1 to 5 ml/min for both KRU and GFR. This demonstrated that predictive equations that use both βTP and β2M had greater accuracy than the best equation using a single protein (β2M) at cut-off levels of measured clearance ranging from 1 to 5 ml/min. This was true for both estimated GFR and KRU. For instance, at a cut-off KRU >2 ml/min/1.73 m^2, the integrated discrimination improvement index was 0.216 for the combined equation compared with 0.171 for the equation using β2M alone ($P = 0.001$). Likewise, at a cut-off GFR >2 ml/min/1.73 m^2 the corresponding values for the integrated discrimination improvement index were 0.200 and 0.125 ($P < 0.001$).

The best modeled equation of GFR (Equation 1) explained 70% of the variance ($R^2 = 0.70$).

$$\textit{Estimated GFR} = \frac{13.471}{\beta TP} + \frac{52.379}{\beta 2M} + \frac{782.909}{\textit{creatinine}} + 0.519 \times \textit{gender factor} - 3.939 \quad \text{Equation 1}$$

Where for gender, male = 1, female = 0

The best model of KRU (Equation 2) explained 63% of the variance ($R^2 = 0.63$).

$$\textit{Estimated KRU} = \frac{9.097}{\beta TP} + \frac{37.568}{\beta 2M} + 0.402 \times \textit{ethnicity factor} - 2.049 \quad \text{Equation 2}$$

Where for ethnicity, Caucasian = 1, Non-Caucasian = 0

Leave-out one-cross validation for estimated GFR and KRU demonstrated a pseudo-R^2 of 0.66 and 0.60, respectively, which were similar to the performance of the above regression equations in the modeling cohort (R^2 = 0.70 and 0.63, respectively).

Evaluation of predictive equations

The best modeled predictive equations for estimating KRU and GFR were compared with measured KRU and GFR (using urinary urea and creatinine clearances) in the modeling and validation cohorts using correlation and Bland-Altman analysis.[26] Level of agreement for different cut-off levels

Table 1 | Baseline characteristics of patients

Descriptive	Modeling group (n = 191)	Validation group (n = 40)	P
Demographics			
Age (y)	67 (IQR 53–77)	68 (IQR 50–77)	0.910
Male (%)	69.6	60	0.235
Dry weight (kg)	73.6 (IQR 64.5–88.5)	85.2 (IQR 68.1–96.4)	0.159
Height (cm)	170 (IQR 161–177)	170 (IQR 163–178)	0.698
BMI	25.4 (IQR 22.6–31.1)	27.6 (IQR 23.0–32.3)	0.205
BSA (m^2)	1.86 (IQR 1.7–2.0)	1.95 (IQR 1.73–2.11)	0.128
Watson volume (l)	38.7 (IQR 34.2–43.2)	40 (IQR 32.3–46.7)	0.355
Ethnicity (%)			0.106
White	73.3	82.5	
Black	8.4	12.5	
Asian	15.2	2.5	
Other	0.5	2.5	
Primary renal disease (%)			0.157
Diabetes	26.7	22.5	
Glomerulonephritis	12.6	27.5	
Polycystic kidney disease	8.4	12.5	
Tubulointerstitial disease	2.6	0	
Hypertension or renovascular disease	16.8	15.0	
Other	33.0	22.5	
Mean weekly systolic BP (mmHg)	150 (IQR 135–162)	152 (IQR 133–171)	0.802
Mean weekly diastolic BP (mmHg)	75 (IQR 66–84)	69 (IQR 63–80)	0.163
KRU (ml/min/1.73 m^2)	1.29 (IQR 0–2.38)	0.62 (IQR 0–1.51)	0.042*
Residual GFR (ml/min/1.73 m^2)	1.72 (IQR 0–3.51)	0.74 (IQR 0–2.02)	0.016*
Interdialytic urine volume (ml)	675 (0–1510)	295 (IQR 0–865)	0.026*
Anuric patients (%)	34.0	42.5	0.309
Diuretic use (%)	30.9	27.5	0.671
Diuretic dose (milligram of furosemide)	0 (IQR 0–80)	0 (IQR 0–40)	0.714
Dialysis parameters			
HDF/high flux HD (%)	82.7/17.3	80/20	0.682
Convective volume (l)	16.7 (IQR 13.1–19.5)	18.2 (IQR 13.4–21.5)	0.191
Ultrafiltration volume (l)	1.7 (IQR 0.98–2.4)	1.8 (IQR 0.9–2.3)	0.986
Mean IDWG (kg)	1.5 (IQR 0.8–2)	1.3 (IQR 0.7–2)	0.655
Dialysis vintage (y)	1.9 (IQR 0.8–4.8)	2.2 (IQR 0.95–4.35)	0.882
Equilibrated Kt/V (dialyzer)	1.1 (IQR 0.9–1.3)	1.2 (IQR 1.0–1.4)	0.194
Total Kt/V (renal + dialyzer)	1.3 (IQR 1.2–1.5)	1.4 (IQR 1.2–1.5)	0.914
Co-morbidity			
Charlson co-morbidity index	4.0 (IQR 2–5)	3.0 (IQR 2–6)	0.623
Presence of atheromatous disease (%)	49.7	52.5	0.751
Presence of malignant disease (%)	8.9	20.0	0.013*
Presence of diabetes (%)	36.6	25.0	0.159
CRP (mg/l)	6 (IQR 5.0–13.0)	10 (IQR 5.0-20.8)	0.019*
βTP (mg/l)	6.51 (IQR 5.34–9.35)	6.86 (IQR 5.86–7.71)	0.909
β2M (mg/l)	24.3 (IQR 19.2–29.1)	24.4 (IQR 20.3–28.4)	0.645

BMI, body mass index (calculated from dry weight); BP, blood pressure; BSA, body surface area (calculated from dry weight); βTP, β-trace protein; β2M, β2-microglobulin; CRP, C-reactive protein; GFR, glomerular filtration rate; HD, hemodialysis; HDF, hemodiafiltration; IDWG, interdialytic weight gain; IQR, interquartile range; KRU, residual urea clearance.
Atheromatous disease indicates the presence of any of the following: coronary artery disease, cerebrovascular disease, renovascular disease and peripheral vascular disease. Diuretic dose is give as milligrams of furosemide. Anuric patients are patients with interdialytic urine volume < 200 ml.
*Denotes statistical significance ($P < 0.05$).

of residual kidney function was assessed using the kappa statistic (κ).

Estimated and measured values of both parameters correlated significantly in both modeling (correlation coefficients for KRU and GFR were 0.781 and 0.801, respectively [$P < 0.001$]) and validation cohorts (correlation coefficient for KRU and GFR were 0.783 and 0.762, respectively [$P < 0.001$]). Mean bias between measured and estimated KRU in the validation cohort was –0.50 ml/min [95% CI –0.25 to –0.75] with 95% limits of agreement from –2.03 to 1.04 ml/min. For GFR, mean bias was –0.64 ml/min [95% CI –0.89 to –0.39] with 95% limits of agreement from –2.84 to 1.57 ml/min (Figure 1).

Level of agreement using the kappa statistic (κ)[27] between the proportions of patients with measured and predicted levels of GFR above cut-offs in the range 1 to 3 ml/min/1.73 m^2 was substantial in the modeling cohort (κ = 0.65–0.67, all $P < 0.001$) and ranged from moderate to substantial in the validation cohort (κ = 0.43–0.77, all $P < 0.01$). Similarly, level of agreement for KRU above cut-offs in the range 1 to 3 ml/min/1.73 m^2 was moderate to substantial in the modeling cohort (κ = 0.51–0.66, all $P < 0.001$) and fair to substantial in the validation cohort (κ = 0.36–0.65, all $P < 0.02$). For both GFR and KRU, the level of agreements deteriorated outside of these ranges.

Table 2 | Determinants of βTP: univariable and multivariable regression analysis (R^2 of multivariable model = 0.552)

	Univariable model			Multivariable model		
Determinant	Beta	Standard error	Significance	Beta	Standard error	Significance
Demographic/ Clinical data						
Age (y)	−0.041	0.013	0.001*	−0.036	0.009	<0.001
Dry weight (kg)	−0.019	0.01	0.069			
Post HD weight (kg)	−0.018	0.01	0.081			
Height (cm)	0.003	0.018	0.886			
BMI (kg/m^2)	−0.041	0.024	0.087			
Male gender	0.610	0.427	0.155	1.394	0.326	<0.001
BSA (m^2)	−1.217	0.831	0.145	−2.205	0.627	<0.001
Watson volume (ml)	−0.00001	0	0.671			
Ethnicity (white)	−1.77	0.427	<0.001*			
Systolic BP (mmHg)	−0.002	0.009	0.806			
Diastolic BP (mmHg)	0.001	0.013	0.932			
GFR (ml/min/1.73 m^2)	−0.813	0.066	<0.001*	−0.8	0.061	<0.001
KRU (ml/min/1.73 m^2)	−1.208	0.1	<0.001*			
Comorbidities						
CCI	−0.14	0.09	0.124			
Atheromatous disease	0.959	0.389	0.015*	0.857	0.283	0.003
Diabetes mellitus	−0.072	0.41	0.861			
Malignancy	−1.835	0.68	0.008*			
CRP	−0.011	0.011	0.35			
Diuretic use	−1.484	0.413	<0.001*			
Diuretic dose	−0.002	0.002	0.179			
Dialysis parameters						
HD Modality (HDF/high-flux HD)	0.559	0.521	0.285	0.896	0.353	0.012
Vintage (y)	0.035	0.026	0.168			
UF volume (l)	0.001	<0.001	<0.001*			
Convective volume (l)	0.024	0.027	0.359			
Dialyser Kt/V	2.462	0.74	0.001*			
Mean IDWG (kg)	0.948	0.224	<0.001*			

BMI, body mass index; BP, blood pressure; BSA, body surface area; βTP, β-trace protein; CCI, Charlson comorbidity index; CRP, C-reactive protein; GFR, glomerular filtration rate; HD, hemodialysis; HDF, hemodiafiltration; IDWG, interdialytic weight gain; KRU, residual urea clearance; UF, ultrafiltration volume.
The diuretic dose is given in milligrams furosemide.
*Denotes statistical significance ($P < 0.05$).

Application of predictive equations for KRU and GFR to KDOQI incremental hemodialysis algorithm

The diagnostic accuracy of predictive equations to identify those with KRU >2 ml/min/1.73 m^2, which might allow safe reduction of minimum dialysis Kt/V targets as suggested in the KDOQI Hemodialysis Adequacy guidelines, was assessed in modeling and validation groups. Receiving operator characteristic analyses were performed for prediction of various cut-off levels of measured GFR or KRU using the prediction equations in both the modeling and validation cohorts. The prediction equations demonstrated a high degree of accuracy with area under curve values between 0.900 and 0.948 (Table 5). For instance, identifying patients in the modeling cohort with measured levels of KRU >2 ml/min/1.73 m^2, using cut-off predicted KRU levels >2 ml/min/1.73 m^2 yielded an area under curve of 0.903, a sensitivity of 58%, and a specificity 92%, while in the validation cohort corresponding values were area under curve 0.948, sensitivity 71%, and specificity 94%.

Using our modeled equation for estimating KRU, we determined the proportion of patients whose minimum target Kt/V could be safely reduced according to KDOQI Hemodialysis Adequacy guidelines.[4] In the modeling cohort, estimated levels of KRU >2 ml/min/1.73 m^2 correctly identified patients with measured values above and below this cut-off level in 81.2% subjects. In 13.6% of the cohort, KRU was falsely estimated to be less than 2 ml/min/1.73 m^2 (false negative) and falsely estimated to be greater than 2 ml/min/1.73 m^2 (false positive) in 5.2%. Among false positives, the mean underestimate between measured KRU and the critical cut-off level of 2 ml/min/1.73 m^2 was 0.75 ml/min/1.73 m^2 (range 0.04–2 ml/min/1.73 m^2). If target standardized Kt/V had been reduced from 1.2 to 0.9 for these patients in accordance with KDOQI guidance,[4,28] this would have resulted in underdialysis equivalent to a mean of 0.11 standardized Kt/V units (range 0.01–0.29). However, 94.8% of patients would have received doses of dialysis at or above target.

In the validation cohort, patients with KRU >2 ml/min/1.73 m^2 were correctly identified in 90% of cases. False-positive rate was 5%. Of these, mean underestimation between measured KRU and the critical cut-off level of 2 ml/min/1.73 m^2 was 0.73 ml/min/1.73 m^2. Applying KDOQI guidance would have resulted in underdialysis in these patients by a mean of 0.11 standardized Kt/V units, though 95% of the cohort would have received a dialysis dose at or above target.

DISCUSSION

βTP has been proposed as a suitable marker of GFR because its extra renal interference is said to be minimal.[10,11,14] We

Table 3 | Determinants of β2M: univariable and multivariable regression analysis (R^2 of multivariable model = 0.484)

	Univariable model			Multivariable model		
Determinant	Beta	Standard error	Significance	Beta	Standard error	Significance
Demographic data						
Age (y)	−0.063	0.037	0.088			
Dry weight (kg)	−0.059	0.029	0.044*			
Post HD weight (kg)	−0.058	0.029	0.045*			
Height (cm)	−0.021	0.052	0.688			
BMI (kg/m^2)	−0.118	0.068	0.085			
Male gender	−2.43	1.223	0.048*			
BSA (m^2)	−4.363	2.383	0.069			
Watson volume (ml)	0	0	0.079			
Race, Caucasian	−2.412	1.272	0.059			
Systolic BP (mmHg)	−0.013	0.026	0.616			
Diastolic BP (mmHg)	0.04	0.039	0.303			
GFR (ml/min/1.73 m^2)	−2.403	0.184	<0.001*	−2.36	0.183	<0.001
KRU (ml/min/1.73 m^2)	−3.476	0.287	<0.001*			
Comorbidities						
CCI	−0.339	0.261	0.195			
Atheromatous disease	1.178	1.133	0.3			
Diabetes mellitus	−3.103	1.157	0.008*	−2.031	0.849	0.018*
Malignancy	0.129	1.995	0.949			
CRP	0.001	0.033	0.976			
Diuretic use	−4.425	1.187	<0.001*			
Diuretic dose	−0.004	0.005	0.417			
Dialysis parameters						
HD modality (high-flux HD/HDF)	−0.597	1.502	0.691			
Vintage (y)	0.16	0.073	0.03*			
UF volume (l)	0.002	0.001	0.003*			
Convective volume (l)	0.126	0.076	0.101			
Dialyzer Kt/V	9.413	2.08	<0.001*			
Mean IDWG (kg)	1.78	0.663	0.008*			

BMI, body mass index; BP, blood pressure; BSA, body surface area; β2M, β2-microglobulin; CCI, Charlson comorbidity index; CRP, C-reactive protein; GFR, glomerular filtration rate; HD, hemodialysis; HDF, hemodiafiltration; IDWG, interdialytic weight gain; KRU, residual urea clearance; UF, ultrafiltration volume.
The diuretic dose is given in milligrams furosemide.
*Denotes statistical significance ($P < 0.05$).

found, though, that age, gender, body surface area, presence of atheromatous disease, and HD modality were independent determinants of βTP. Prevalence of atheromatous disease and male gender were positively related, and use of HDF inversely related consistent with previous reports.[9,29] For β2M, we found an association only with RKF and diabetic status. Although β2M clearance is superior in HDF, HD modality was not an independent determinant of serum β2M levels.[17,20] The high prevalence of significant RKF in this cohort, a more important determinant of β2M levels than even convective clearance, may be a factor.[20]

Predictive equations for GFR and KRU correlated well with measured GFR and KRU. The integrated discrimination improvement index demonstrated superior diagnostic ability when both βTP and β2M were incorporated into regression models. The best constructed regression equation using both biomarkers could explain 63% and 70% of KRU and GFR variance, respectively; however, a substantial amount of variation still remains unexplained. Mean bias between measured and estimated parameters of RKF was –0.5 ml/min for KRU and –0.64 ml/min for GFR, with wide limits of agreement. Our findings suggest that equations incorporating serum levels of βTP and β2M may not be accurate enough to estimate GFR if RKF were to be used to calculate minimum HD targets as advocated by Gotch[30] and Casino and Lopez.[5,28,31] KDOQI guidelines propose an alternative, relatively simple approach for including RKF into HD prescription. This confines attempts to reduce dialysis dose to patients with a KRU of 2 ml/min/1.73 m^2 or more, assuming RKF to be absent below this.[4] We have examined whether equations incorporating βTP and β2M could be used to accurately distinguish patients with KRU >2 ml/min/1.73 m^2, thus allowing safe implementation of the KDOQI algorithm.

Applying our modeled equations to estimate KRU at cut-off >2 ml/min/1.73 m^2 in the validation cohort demonstrated substantial agreement with measured KRU ($\kappa = 0.654$). Our modeled equations incorrectly estimated KRU to be ≥2 ml/min/1.73 m^2 in 5.0% to 5.2% of patients, suggesting that only a small proportion of patients would receive underdialysis if KRU estimates were used to set minimum dialysis Kt/V targets according to the KDOQI algorithm.

There were a number of limitations to our study. The number of patients in validation cohorts was relatively small (n = 40). Modeled equations based on a small sample size may limit applicability to the general population because other co-morbid factors, such as inflammation, active lupus, and malignancy, may affect levels of βTP[9] and β2M.[21,22] Similarly, the modeled regression equations were based on a relatively homogenous population with a high proportion treated with HDF. Our findings may not apply to other

Table 4 | Linear regression equations for KRU and GFR

Model	Biomarker used	Parameters	β coefficient	Significance	R^2
KRU	βTP	1/βTP	14.985	<0.001	0.569
		1/creatinine	682.73	<0.001	
		1/urea	11.421	0.03	
		Male gender	0.521	0.001	
	β2M	1/ β2M	50.022	<0.001	0.597
		1/creatinine	596.149	<0.001	
		1/urea	−14.618	0.004	
		Caucasian ethnicity	0.483	0.003	
	βTP and β2M	1/βTP	9.097	<0.001	0.625
		1/β2M	37.568	<0.001	
		Caucasian ethnicity	0.402	0.01	
GFR	βTP	1/βTP	23.968	<0.001	0.633
		1/creatinine	1230.716	<0.001	
		Age	−0.016	0.019	
		Gender	0.938	<0.001	
	β2M	1/β2M	78.247	<0.001	0.665
		1/creatinine	1143.816	<0.001	
		1/urea	−20.4	0.003	
		Caucasian ethnicity	0.469	0.033	
	βTP and β2M	1/βTP	13.471	<0.001	0.700
		1/β2M	52.379	<0.001	
		1/creatinine	782.909	<0.001	
		Male gender	0.519	0.012	

βTP, β-trace protein; β2M, β2-microglobulin; GFR, glomerular filtration rate; KRU, residual urea clearance.

patient cohorts of different ethnic mix, body composition, and HDF prevalence. We have used the arithmetic mean of pre- and post-levels of urea and creatinine because this is a commonly used method for calculating GFR and KRU. It does, however, risk potential inaccuracies related to the non-linear interdialytic increments of both urine excretion and the plasma solute levels.

βTP and β2M measurements were carried out using nephelometric and turbidimetric techniques, respectively. Alternative methods are available for both proteins,[32,33] and variation between different assays may therefore limit applicability of our equations. Additionally, although the precision of both assays seems robust in non-uremic serum, we cannot exclude the potential interference of the assay by toxins related to advanced uremia or the dialysis procedure; however, the manufacturer does not preclude reporting of these analytes in uremic samples. Both βTP and β2M are removed during high-flux HD and HDF, and the concentrations of both increase during the interdialytic period. β2M levels exceed 95% of predialysis levels 44 hours after session end.[13] It is likely, therefore, that the predialysis levels after the long interdialytic gap will equal or exceed the previous predialysis value. We know of no comparable data for βTP, but, by the same logic, the level after the long gap is likely to be the most indicative of peak levels. Hence levels of these biomarkers immediately before the first dialysis of the week are likely to be the most suitable for predicting RKF. Levels at other times may overestimate RKF. The levels of both these parameters will also vary according to the volume status of these patients but to a lesser extent.

Serial β2M levels increase with declining RKF,[34] and though there are no comparable data for βTP, a similar relationship would be expected. However, our modeled prediction equations were developed using β2M, βTP, and RKF measurements at a single time point. Hence the equations may not perform similarly in predicting progressive loss of RKF from serial levels as would be the case if there were differences in the relative rates of change of B2M and βTP levels with progressive loss of RKF. Further work is required to examine this issue before clinical application of our findings. Finally, measurement of GFR using interdialytic urine collections may be prone to error, and ideally the regression equations should be validated against a gold-standard method of GFR measurement such as ^{125}I-iothalamate or chromium-51 labeled ethylenediamine tetraacetic acid. However, these techniques are impractical for routine clinical use, and the primary objective of this investigation was to determine whether equations involving βTP and/or β2M could replace standard measurements of GFR and KRU using interdialytic urine collections.

In summary, serum levels of βTP and β2M are reasonable indicators of RKF. Inclusion of both into regression equations can provide a better estimate of RKF than either molecule alone. However, serum levels of βTP and β2M may not be accurate enough to replace the standard estimation of GFR using urea and creatinine clearances for HD units practicing an incremental HD regime, although the predictive equations using βTP and β2M could potentially be used to identify

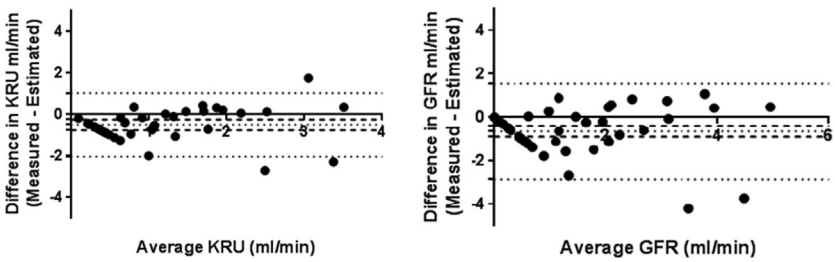

Figure 1 | Bland-Altman analysis of measured versus estimated residual urea clearance (KRU) and glomerular filtration rate (GFR) in validation cohort (dotted lines represent mean bias with 95% limits of agreement, and hashed lines represent 95% confidence interval of mean bias).

Table 5 | Receiver operating characteristic analyses for identification of patients with RKF above defined levels

Population	RKF measure (ml/min/1.73 m^2)	n	Cut-off RKF level to be identified (ml/min/1.73 m^2 of GFR or KRU as appropriate)	AUC	*P*	Sensitivity at predicted cutoff (%)	Specificity at predicted cutoff (%)
Modeling	GFR	114	>1	0.909	<0.001	94	69
		89	>2	0.908	<0.001	76	88
		58	>3	0.941	<0.001	72	92
		42	>4	0.937	<0.001	55	97
		22	>5	0.930	<0.001	59	97
	KRU	104	>1	0.906	<0.001	88	78
		62	>2	0.903	<0.001	58	92
		32	>3	0.900	<0.001	50	96
		10	>4	0.930	<0.001	30	99
Validation	GFR	17	>1	0.903	<0.001	94	52
		10	>2	0.910	<0.001	70	77
		4	>3	0.944	0.004	100	94
	KRU	14	>1	0.942	<0.001	100	69
		7	>2	0.948	<0.001	71	94

AUC, area under curve; GFR, glomerular filtration rate; KRU, residual urea clearance, RKF, residual kidney function.
In these receiving operator characteristic analyses, the cut-off level of measured GFR or KRU to be identified is shown in column 2. Identification of patients with GFR/KRU above these levels is with predicted KRU/GFR from equations (1) and (2) above this same level.

those with KRU > 2 ml/min/1.73 m^2 to follow the suggested KDOQI incremental HD algorithm. Validation of this approach in larger, more diverse cohorts of patients is required.

MATERIALS AND METHOD

Ethical approval

The study was approved by the East Midlands National Research Ethics Committee. Informed consent was obtained from all studied patients.

Overall study design

This is a single-center cross-sectional study of prevalent patients undergoing outpatient HD at the East & North Hertfordshire NHS Trust. The center deploys an incremental approach to dialysis prescription involving monthly urine collections while patients continue to pass urine. Around 80% are treated by HDF, the remainder using high-flux HD. All were clinically stable at the time of the study. Patients with positive HIV status and active hepatitis infection were excluded. Two hundred thirty-one patients (n = 231) were recruited. Recruitment was carried out prospectively to ensure that around two thirds of the total cohort had RKF. Patients who produced less than 200 ml of urine volume over the interdialytic period were considered to have no RKF—clearances were not measured in these patients. Of the total cohort, 191 were randomly assigned to the modeling group for derivation of the predictive equations and the remaining 40 were assigned to the validation group.

βTP, β2M, KRU, and GFR, calculated from the mean urea and creatinine clearance, were measured in all patients. Demographic, clinical, and dialysis data including age, gender, Charlson co-morbidity index, dialysis modality, Kt/V, and C-reactive protein were also collected.

Measurement of residual kidney function: mean urea and creatinine clearance

Blood was sampled at the end of the first dialysis session of the week and immediately before the next session. Between these samples, urine was collected over the whole interdialytic period. GFR was estimated as mean of the urea and creatinine clearances. These clearances were calculated using the formula:

$$Clearance(ml/min) = \frac{2 \cdot (U_{ID} \cdot V_{ID})}{t_{ID} \cdot \left(C_{post_{HD1}} + C_{pre_{HD2}}\right)}$$

Where U_{ID} = urinary concentration, V_{ID} = urine volume, t_{ID} = collection duration, $C_{postHD1}$ = plasma concentration at the end of the first dialysis session, and C_{preHD2} = plasma concentrations is the plasma concentration immediately before the start of the second dialysis session. Post-dialysis urea and creatinine measurements were adjusted for rebound using the Smye formula[35] in accordance with the European Best Practice Hemodialysis guidelines.[5]

Measurement of serum β-trace protein and β2-microglobulin

Blood samples for serum βTP and β2M were taken pre-dialysis immediately before the first HD session of the week. Serum βTP was measured with particle-enhanced immune-nephelometric assay (N Latex βTP assay; Siemens Diagnostics, Newark, DE, USA), and serum β2M was measured using by immune-turbidimetric analysis (Olympus AU640; Beckman-Coulter, Brea, CA). Manufacturer-supplied data showed that coefficient of variation for βTP and β2M assays were <6.1% and <10%, respectively.[36,37] As judged by external quality assessment, coefficient of variation for the β2M assay during the period of study was 3.9% at a mean concentration of 3 mg/L and 4.5% at a mean concentration of 6.6 mg/L. For βTP, coefficient of variation was 3.8% at a mean concentration of 0.826 mg/L and 2.6% at a mean concentration of 12.4 mg/L.

Statistical analysis

Data are reported as medians with interquartile ranges (IQR). Comparisons between groups were made using the Mann-Whitney *U* test. For categorical data, the chi-square test was used to assess group differences.

Determining independent predictors of βTP and β2M

Linear regression analysis was used to identify significant determinants of βTP and β2M. Potentially significant demographic, clinical, and dialysis parameters were entered into the regression model to determine the most significant predictors

of the dependent variable (βTP or β2M) in univariable and multivariable analyses. Variables with a significance level <0.05 were considered significant.

Construction and validation of prediction equations to estimate parameters of RKF

Prediction equations for KRU and GFR based on βTP alone, β2M alone, and both biomarkers together along with relevant covariates were constructed using linear regression modeling. Independent variables were examined for multi-collinearity. All independent variables had a variance inflation factor <3, suggesting minimal collinearity in the regression models. Residual plots were inspected for normality and homoscedasticity. The Integrated Discrimination Improvement index[25] was used to assess the predictive accuracy of the equation that incorporated both biomarkers over the best model using a single protein for cut-off levels 1 to 5 ml/min for both KRU and GFR. To assess for potential overfitting of regression models for estimated GFR and KRU, leave-out one cross validation was applied to the entire cohort (modeling plus validation) to calculate pseudo-R^2 for the predictive equations.

Evaluation of predictive equations for KRU and GFR

Correlation was between measured and estimated (from equations) for KRU and GFR using Spearman's correlation coefficient. Bland-Altman[26] analysis was used to compare measured and estimated KRU and GFR in the validation cohort. Level of agreement between measured and predicted KRU and GFR at different cut-off levels was assessed using the kappa statistic.[27] Receiving operator characteristic analysis was performed for prediction of various cut-off levels of measured GFR and KRU using prediction equations in both modeling and validation cohorts.

DISCLOSURE

All the authors declared no competing interests.

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