ENDOGENOUS MARKERS FOR ESTIMATION OF RENAL FUNCTION IN PERITONEAL DIALYSIS PATIENTS

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◆ **Objective:** This method comparison study, conducted at the peritoneal dialysis (PD) outpatient clinic of the Department of Renal Medicine, Aarhus University Hospital, Denmark, set out to evaluate the accuracy and reproducibility of methods for estimating glomerular filtration rate (GFR) based on endogenous markers in PD patients.

◆ **Patients:** The 12 consecutive patients included in the study were examined twice while in a stable condition. All patients finished the study. Inclusion criteria were age 18 years or older, ability to collect 24-hour urine, and urine production greater than 300 mL in 24 hours.

◆ **Main Outcome Measures:** The methods for estimating GFR using endogenous markers included the average of urinary clearances of creatinine and urea [U-Cl(crea-urea)] and two equations using the serum concentration of cystatin C [eGFR(CysC)]. The resulting GFR estimates were compared with those obtained using urinary and corrected plasma clearances of ⁵¹Cr-EDTA [U-Cl(EDTA) and cP-Cl(EDTA)], the corrected plasma clearance being plasma clearance minus dialysate clearance.

◆ **Results:** Compared with the U-Cl(EDTA), the U-Cl(crea-urea) GFR estimate was 12% higher [95% confidence limits (CL): 3%, 21%]. Although significantly different ($p = 0.01$), the latter two methods showed the best agreement. The estimates obtained using the eGFR(CysC) methods were skewed from $y = x$ compared with the estimates obtained using other methods, indicating strong bias, probably because of extrarenal elimination. The cP-Cl(EDTA) estimate was 34% (95% CL: 26%, 42%), higher than the U-Cl(EDTA) estimate ($p < 0.001$). The reproducibility (coefficients of variation) differed significantly between methods: cP-Cl(EDTA), 7%; U-Cl(EDTA), 14%; U-Cl(crea-urea), 18%; and both eGFR(CysC) methods, 3%.

◆ **Conclusions:** In PD patients, GFR may be estimated as U-Cl(crea-urea) when complete urine collection is performed, taking into account an overestimation of approximately 12%. The available equations for eGFR(CysC) seem to be inaccurate; further development and validation is desirable. Omitting the eGFR(CysC) methods, cP-Cl(EDTA) was the most reproducible method and might be useful in certain situations.

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Preservation of residual renal function (RRF) in peritoneal dialysis (PD) patients is associated with decreased morbidity and mortality, independent of dialysis dose (1–4). Thus, reliable and validated methods for estimation of the glomerular filtration rate (GFR) in PD patients are greatly needed.

The most reliable methods for the estimation of GFR involve an exogenous marker. After injection of ⁵¹Cr-EDTA, for example, GFR can be measured using a plasma clearance technique based on blood samples (5,6). However, the former method is labor-intensive and expensive, and data in PD patients are scarce.

In clinical practice, guidelines recommend that GFR be estimated using the average of creatinine and urea clearances based on a 24-hour urine sample [U-Cl(crea-urea)]; however, handling of those substances in the renal tubules influences the result. Moreover, urine collection may be incomplete and even impossible to obtain in some patients.

Estimated GFR based on plasma creatinine [eGFR(crea)] and no urine sampling is increasingly used in clinical practice. However, dialysis removes creatinine, and the plasma concentration of creatinine does not

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solely depend on GFR, thus making eGFR(crea) useless in PD patients.

Serum cystatin C (CysC) was proposed to be a marker of RRF in PD patients (7,8). Two equations based on serum CysC [eGFR(CysC)] for use in dialysis patients were developed: one from Hoek and colleagues (9) for all dialysis patients, and another from Yang and colleagues (10) for patients on continuous ambulatory PD. The eGFR(CysC) proposed by Hoek was tested in 28 PD patients (11). Compared with U-Cl(crea-urea), the eGFR(CysC) results did not differ significantly. However, compared with 7-hour ^{51}Cr -EDTA plasma clearance, eGFR(CysC) showed a mean bias of -20%. The equation proposed by Yang has not been externally validated.

The aim of the present study was to compare estimated GFR based on U-Cl(crea-urea) and the CysC-based equations with clearances determined using ^{51}Cr -EDTA in PD patients. We also investigated the week-to-week reproducibility of GFR estimates, and we propose standard deviations for power calculations to be used in clinical studies on the preservation of RRF.

METHODS

SUBJECTS

We recruited 12 PD patients from the outpatient clinic at the Department of Renal Medicine. Patients were eligible for inclusion if they were able to perform a 24-hour urine collection, had a 24-hour urine volume greater than 300 mL, were 18 years of age or older, had not changed medication or dialysis treatment within the preceding 4 weeks, and were free of ongoing infection or acute illness. Eligible patients were enrolled consecutively until 12 patients were included. Two patients changed dialysis bags manually and were dialyzed continuously. Ten patients used an automated cyclor at night. Of the latter patients, 6 also had dialysis fluid in the abdomen during the daytime. All patients provided written informed consent. The Danish Data Protection Agency and the Central Denmark Region Committees on Biomedical and Research Ethics approved the study (M-20070212), which was conducted in accordance with the Helsinki Declaration of 1975 as revised in 2000.

STUDY PROTOCOL

Patients visited the research clinic on a random day. Individual PD prescriptions were not changed. Weight and height were measured, and the patient rested for

at least 20 minutes. An hour 0 blood sample was drawn, and a bolus of 3.7 MBq ^{51}Cr -EDTA (GE Healthcare Limited, Hammersmith, UK) was injected into a cubital vein. Blood samples were drawn from a cubital vein at 3, 5, and 24 hours using new cannulas. Urine and dialysate were correspondingly collected over approximately 24 hours.

Patients were requested to eat and drink according to usual recommendations, and they were allowed to be moderately physically active during the study period. The procedure was repeated 4 – 10 days later.

Endogenous substances: Blood samples drawn at 3 hours were analyzed for creatinine, urea, and CysC.

Creatinine was analyzed in plasma and urine using an enzymatic method calibrated to be traceable to an isotope dilution mass spectrometry reference measurement procedure (Vitros: Ortho-Clinical Diagnostics, Cardiff, UK). Coefficients of variation (CVs) on analyses performed the same day were 1.3% at plasma creatinine = 451 $\mu\text{mol/L}$, 0.9% at plasma creatinine = 1159 $\mu\text{mol/L}$, 1.4% at urinary creatinine = 5441 $\mu\text{mol/L}$, and 0.9% at urinary creatinine = 7320 $\mu\text{mol/L}$.

A colorimetric method was used for analysis of urea in plasma and urine (Vitros). On analyses performed the same day, the CVs were 1.2% at plasma urea = 13.8 mmol/L and 1.2% at urinary urea = 267 mmol/L.

Serum CysC was analyzed using a particle-enhanced nephelometric immunoassay [Siemens N Latex cystatin C assay on the Behring Nephelometer II: Siemens Healthcare Diagnostics Products (formerly Dade-Behring), Marburg, Germany]. For our CysC double measurements, the CV was 2.7%. In the two studies proposing equations for GFR estimation (9,10), CysC had been measured using the same method.

^{51}Cr -EDTA: For the ^{51}Cr -EDTA analysis, blood samples drawn at 5 and 24 hours were used, as previously reported (6,12,13). Dialysate collection ended at the last exchange of dialysate before blood sampling at 24 hours; in most patients, this collection occurred in the morning. Dialysate collection periods (z) were thus not exactly 24 hours. Dialysate volumes (V) were measured manually in continuous PD patients and electronically in PD patients using a cyclor (ProCard: Baxter Healthcare Corporation, Deerfield, IL, USA).

Tubes containing plasma, urine, or dialysate were counted in a gamma-ray scintillation detector (Packard Cobra II: PerkinElmer, Waltham, MA, USA) to a statistical accuracy of 1%. The counts per minute were used for calculation of the clearance curves of ^{51}Cr -EDTA. Plasma clearance [P-Cl(EDTA)], dialysate clearance

[D-Cl(EDTA)], and urinary clearance [U-Cl(EDTA)] were assessed using these equations:

$$P\text{-Cl(EDTA)} = \frac{Q_0}{\int_0^{\infty} P(t) dt},$$

$$D\text{-Cl(EDTA)} = \frac{D_{51\text{Cr-EDTA}} * V_{\text{dialysate}}}{\int_0^z P(t) dt}, \text{ and}$$

$$U\text{-Cl(EDTA)} = \frac{U_{51\text{Cr-EDTA}} * V_{\text{urine}}}{\int_0^{24} P(t) dt},$$

where Q_0 is the injected dose of $^{51}\text{Cr-EDTA}$; $P(t)$ is the plasma concentration in the period from 0-infinity, from 0-z, or 0-24 hours; $D_{51\text{Cr-EDTA}}$ is the concentration of $^{51}\text{Cr-EDTA}$ in dialysate; $V_{\text{dialysate}}$ is the volume of dialysate; $U_{51\text{Cr-EDTA}}$ is the concentration of $^{51}\text{Cr-EDTA}$ in urine; and V_{urine} is the volume of urine.

For estimations of RRF, plasma clearance must be corrected for dialysate clearance:

$$cP\text{-Cl(EDTA)} = P\text{-Cl(EDTA)} - D\text{-Cl(EDTA)}.$$

Creatinine and urea clearances in urine [U-Cl(x)] and dialysate [D-Cl(x)] were calculated as

$$U\text{-Cl}(x) = \frac{U(x) * V_{\text{urine}}}{P(x) * \text{time}}$$

and

$$D\text{-Cl}(x) = \frac{D(x) * V_{\text{dialysate}}}{P(x) * \text{time}}$$

where $U(x)$ is the concentration in urine, $P(x)$ the concentration in plasma, and $D(x)$ is the dialysate concentration. The V_{urine} and $V_{\text{dialysate}}$ are the volume of urine and dialysate respectively. Time is expressed in minutes.

Cystatin C: The eGFR(CysC) was calculated as

$$e\text{GFR(CysC)} = -0.7 + \frac{22}{\text{CysC}} \quad (\text{Hoek})$$

and

$$e\text{GFR(CysC)} = (6.736 - 0.566 * \text{CysC})^2 * 0.25 + (6.736 - 0.566 * \text{CysC})^{-2} * 0.25 - 0.5 \quad (\text{Yang}),$$

where the CysC unit is milligrams per liter.

Body surface area was calculated using the Du Bois and Du Bois formula (14), and all clearances and GFR estimations are presented in milliliters per minute per 1.73 m² body surface area.

STATISTICAL ANALYSES

To check for normal distribution of data, Q-Q plots were used. All statistical analyses were performed on log-transformed data.

A Bland-Altman analysis was used to examine pairwise agreement within and between methods (15). For each method, the measurement errors at visit 1 and visit 2 were compared using a Pitman test, and the standard deviations (SDs) on the differences between visit 1 and visit 2 were transformed into a method-specific CV (16,17).

To manage the problem of multiple p values calculated for each method on the same set of patients, the univariate statistical analyses were supplemented with a multivariate analysis to compare visits 1 and 2 (Hotelling T-squared statistic) (18). Furthermore, a comparison of method-specific measurement errors was made using a test for compound symmetry in the multivariate analysis.

A p value less than 0.05 was regarded statistically significant. Statistical analyses were performed in Stata/IC 11.1 (StataCorp LP, College Station, TX, USA).

RESULTS

Table 1 shows the characteristics of the study patients.

At visit 2, radioactivity counts per minute in the hour 0 samples were higher than background radiation in some patients because of a residual amount from the $^{51}\text{Cr-EDTA}$ injected at visit 1. This residual at hour 0 was adjusted for clearance and subtracted from the total counts per minute.

REPRODUCIBILITY

Urine volumes and urinary excretion of creatinine had almost the same magnitude at both visits (Figure 1).

For each of the 5 methods used to estimate GFR, the measurement errors did not change from visit 1 to visit 2, $p > 0.05$ (Pitman test), indicating stable performance.

Measured cP-Cl(EDTA) was significantly higher at visit 1 than at visit 2, $p = 0.01$ (Bland-Altman analysis). A multivariate analysis (Hotelling T-squared statistic)

TABLE 1
Characteristics of the 12 Peritoneal Dialysis Patients

Variable	Value	
	Visit 1	Visit 2
Sex (women/men)	4/8	
Age (years)		
Median	62	
Range	44–85	
Dialysis duration (months)		
Median	22	
Range	3–54	
Primary cause of ESRD (n)		
Diabetes	3	
Polycystic kidney disease	3	
Hypertension	1	
Lithium-induced nephropathy	1	
Reflux-induced nephropathy	1	
Unknown	3	
Clinical characteristics		
Weight (kg)		
Median	72.6	73.0
Range	52.5–109.9	52.4–112.4
Body-mass index (kg/m ²)		
Median	25.3	—
Range	21.2–33.2	—
Blood pressure		
[systolic/diastolic (mmHg)]		
Median	145/90	—
Range	98–210/65–110	—
Ultrafiltration (mL)		
Median	928	743
Range	–271 to 1422	141–1503
Plasma creatinine (μmol/L)		
Median	728	777
Range	184–1004	185–995
Plasma urea (mmol/L)		
Median	16.7	17.9
Range	8.3–30.5	10.7–27.8
Serum cystatin C (mg/L)		
Median	4.09	4.08
Range	1.82–5.40	2.00–5.53
Urine volume (mL/24 h)		
Median	1322	1568
Range	488–2063	370–2066

ESRD = end-stage renal disease.

based on all methods showed a trend in the same direction, $p = 0.07$. The Bland–Altman plots showed acceptable variability with a general trend toward a positive difference (Figure 2). The differences between visits were quite small (Table 2), and the averaged values at visit 1 and visit 2 were used to compare methods.

Figure 1 — Urine output (closed circles) and urinary creatinine excretion (open circles) at visit 1 and visit 2.

As shown in Table 1, PD patients often have a substantial day-to-day variation in ultrafiltration volume. However, dialysate clearances (Table 2) were similar at visit 1 and visit 2 ($p = 0.86$), and measurement errors did not differ between the methods ($p = 0.49$).

Measurement errors for each method were compared using a test for compound symmetry in a multivariate analysis of covariances. Overall, the measurement errors and, therefore, also the limits of agreement (LOA) and CVs, differed significantly, $p < 0.001$.

The SD of the difference between measurements [SD(diff), Table 2] can be used for power calculations if a study is designed for paired measurements. If the SD on a single measurement is of interest, the SD(diff) should be divided by the square root of 2 (19). Table 2 sets out the number of patients needed to detect a 10% change in GFR.

COMPARISON OF METHODS

Table 3 presents the statistical results of the log-transformed data.

Compared with log-transformed data, non-transformed data are easier to relate to clinical practice, and non-transformed data were therefore chosen for visualization. Figure 3 depicts the averaged values at visit 1 and visit 2 in pairwise comparisons.

Visually, U-Cl(crea-urea) and U-Cl(EDTA) seemed to be very close to each other when $\text{GFR} < 10 \text{ mL/min/1.73 m}^2$. Moreover, eGFR(CysC) by Hoek and by Yang were skewed from $y = x$ when compared with clearance of both $^{51}\text{Cr-EDTA}$ and U-Cl(crea-urea) (Figure 3).

Figure 2 — Bland–Altman plots for each method of estimating glomerular filtration rate (GFR), based on log-transformed data. Original unit was milliliters per minute per 1.73 m² body surface area. eGFR(CysC) = cystatin C–based equation for estimated GFR; cP-Cl(EDTA) = corrected ⁵¹Cr-EDTA plasma clearance; U-Cl(EDTA) = ⁵¹Cr-EDTA urine clearance; U-Cl(crea-urea) = averaged creatinine and urea clearance.

TABLE 2
Medians and Differences in Peritoneal Dialysis Patients at Visit 1 and Visit 2 Distributed According to Method

Method	Median ^a (mL/min/1.73 m ²)	Range	Difference ^b (%)	95% CL (%)	LOA (%)	CV (%)	SD(diff)	Sample size ^c
cP-Cl(EDTA)	5.4	1.1–24.0	9	3, 16	–11, 30	7	0.105	43
U-Cl(EDTA)	4.1	0.7–18.6	3	–10, 15	36, 41	14	0.198	150
U-Cl(crea-urea)	4.3	1.2–21.5	12	–5, 28	–38, 62	18	0.256	250
eGFR(CysC) Hoek	4.7	3.3–10.8	2	–1, 5	–7, 11	3	0.045	4
eGFR(CysC) Yang	4.4	2.8–7.5	3	–0, 5	–5, 11	3	0.041	3
D-Cl(EDTA)	2.8	1.4–4.2	–6	–19, 8	–48, 36	15	0.214	—
D-Cl(crea)	2.7	1.6–3.5	–5	–17, 8	–40, 31	13	0.181	—
D-Cl(urea)	4.2	2.8–5.2	–4	–14, 6	–33, 25	10	0.145	—

cP-Cl(EDTA) = ⁵¹Cr-EDTA plasma clearance with ⁵¹Cr-EDTA dialysate clearance subtracted; U-Cl(EDTA) = ⁵¹Cr-EDTA urine clearance; U-Cl(crea-urea) = averaged creatinine and urea clearance in urine; eGFR(CysC) = cystatin C–based equation for estimated glomerular filtration rate; D-Cl(EDTA) = ⁵¹Cr-EDTA dialysate clearance; D-Cl(crea) = creatinine clearance in dialysate; D-Cl(urea) = urea clearance in dialysate.

^a Calculated using the averages of visit 1 and visit 2 without log transformation.

^b Calculated as visit 1 minus visit 2, with 95% confidence limits (CL), limits of agreement (LOA), coefficient of variation (CV), and standard deviation of the differences [SD(diff)] between visit 1 and visit 2 calculated using Bland–Altman analyses on log-transformed data.

^c The number of patients needed in each of two groups to detect a 10% between-group difference in glomerular filtration rate in a follow-up study.

Urine volume was not related to U-Cl(EDTA), U-Cl(crea-urea), or eGFR(CysC) (Figure 4).

DISCUSSION

In patients with end-stage renal failure, precise and accurate GFR estimations are needed both when the necessity for PD is questioned and when treatment dose of a pharmaceutical that is excreted by the kidney is calculated. Furthermore, reliable GFR measurements can substantially reduce the number of patients needed to obtain significant differences in research studies on preservation of RRF.

In the present study, the eGFR(CysC) range was narrow and showed very small differences between the two visits (Figure 2). However, the eGFR(CysC) results obtained with the Hoek and Yang equations were skewed compared with the results from the other methods (Figure 3, bottom two rows). Despite low SDs, those findings

may indicate severe built-in problems for the CysC-based equations investigated.

Since 1985, CysC has been proposed to be a marker of GFR (20). However, CysC is still not fully investigated, and knowledge of CysC kinetics in humans is especially scarce. Because CysC is catabolized in the renal tubules with virtually no urinary excretion of CysC in healthy subjects (21), clearance of CysC cannot be measured using urinary excretion divided by serum concentration. The suggestion is that estimations of GFR be based on serum concentrations alone. Moreover, an increase in urinary CysC is seen in patients with functional and structural tubular impairment, independent of GFR (22).

Yang *et al.* proposed an equation for GFR estimation in continuous ambulatory PD patients based on CysC values in serum, and Hoek *et al.* proposed equations for PD and hemodialysis patients separately and an equation suitable for all dialysis patients (9,10). From the common eGFR(CysC) equation of Hoek, based on the

TABLE 3
Comparing Clearances of Endogenous Substances to Urinary and Corrected Plasma Clearances of ^{51}Cr -EDTA in the 12 Peritoneal Dialysis Patients^a

		cP-Cl(EDTA)	U-Cl(EDTA)	U-Cl(crea-urea)
U-Cl(EDTA)	Bias (%)	34		
	95% CL (%)	26, 42		
	<i>p</i> Value	<0.001		
	SD(diff)	0.127		
	LOA (%)	9, 59		
U-Cl(crea-urea)	Bias (%)	22	-12	
	95% CL (%)	12, 32	-21, -3	
	<i>p</i> Value	0.001	0.012	
	SD(diff)	0.164	0.139	
	LOA (%)	-10, 54	-39, 15	
eGFR(CysC) ^b Hoek	Bias (%)	10	-24	-12
	95% CL (%)	-19, 40	-58, 10	-41, 18
	<i>p</i> Value	0.453	0.152	0.404
	SD(diff)	0.462	0.532	0.460
	LOA (%)	-80, 101	-128, 80	-102, 79
eGFR(CysC) ^b Yang	Bias (%)	23	-11	1
	95% CL (%)	-8, 54	-47, 25	-31, 33
	<i>p</i> Value	0.136	0.505	0.946
	SD(diff)	0.491	0.562	0.498
	LOA (%)	-73, 119	-121, 99	-97, 99

cP-Cl(EDTA) = ^{51}Cr -EDTA plasma clearance with ^{51}Cr -EDTA dialysate clearance subtracted; U-Cl(EDTA) = ^{51}Cr -EDTA urine clearance; U-Cl(crea-urea) = averaged creatinine and urea clearance in urine; CL = confidence limits; SD(diff) = standard deviation of the differences between visit 1 and visit 2; LOA = limits of agreement; eGFR(CysC) = cystatin C-based equation for estimated glomerular filtration rate.

^a Blood samples were drawn 3 hours after injection of ^{51}Cr -EDTA. Methods were compared pairwise using Bland-Altman analysis.

^b Comparisons including eGFR(CysC) should be interpreted cautiously because the slope of the regression line comparing eGFR(CysC) with ^{51}Cr -EDTA clearances and U-Cl(crea-urea) is skewed from line of identity ($x = y$, Figure 3).

Figure 3 — Pairwise relations between estimations of glomerular filtration rate (GFR). All clearances are the averages of visit 1 and visit 2 and are given in milliliters per minute per 1.73 m² body surface area. Open circles and dotted regression line: 10 patients with glomerular filtration rate less than 10 mL/min/1.73 m². Small boxes: All patients (x-axis and y-axis are scaled 0 – 35). Closed circles and solid regression line: All 12 study patients. Dashed line: line of identity, $y = x$. U-Cl(EDTA) = ⁵¹Cr-EDTA urine clearance; cP-Cl(EDTA) = corrected ⁵¹Cr-EDTA plasma clearance; U-Cl(crea-urea) = averaged creatinine and urea clearance; eGFR(CysC) = cystatin C-based equation for estimated GFR.

Figure 4 — Association between urine volume and clearance measurements (given in milliliters per minute per 1.73 m² body surface area). U-Cl(EDTA) = ⁵¹Cr-EDTA urine clearance; U-Cl(crea-urea) = averaged creatinine and urea clearance; eGFR(CysC) = cystatin C-based equation for estimated glomerular filtration rate.

assumption of an inverse relationship, serum CysC would be 31 mg/L if GFR is zero. The Yang equation is based on the more complex hyperbolic sine function and predicts CysC to reach 10 mg/L when GFR is close to zero. The two equations differ mainly at the extremes, explaining why the results obtained in the present study are quite similar (Figure 5). Studies in anuric humans and rats have demonstrated that serum CysC rarely exceeds 10 mg/L (23,24), favoring the Yang equation, but that equation cannot determine the relationship between CysC and GFR if GFR is greater than 11 mL/min/1.73 m². None of the proposed equations seems to fully cover the GFR range of interest.

In nephrectomized rats, CysC concentrations reached a plateau in less than 24 hours and did not rise further, but creatinine concentrations rose continuously to the end of the experiment (24). That finding could be a result of a lower production rate of CysC in the advanced uremic milieu or by increased extrarenal elimination, or both. Neither Yang nor Hoek validated their equations by comparison with a substance freely filtered and not handled in the tubules. Hoek (9) suggested that U-Cl(crea-urea), used as the reference, was to blame for the disagreements between eGFR(CysC) and U-Cl(crea-urea). However, in our study, both eGFR(CysC) methods showed the same variation when compared with GFR measured by plasma or renal ⁵¹Cr-EDTA clearance, suggesting a problem with the eGFR(CysC) equations or with CysC as a marker of renal function at a low GFR in

Figure 5 — Association between serum cystatin C and estimated glomerular filtration rate (eGFR, given as milliliters per minute per 1.73 m² body surface area) based on two different equations. Solid line: associations proposed by Hoek (9). Dashed line: associations proposed by Yang (10). Dotted lines: range of cystatin C in the study patients.

our PD population, or both. We found that eGFR(CysC) produced underestimates and overestimates at high and low GFR levels respectively (Figure 3). Moreover, we were not able to corroborate the relation between eGFR(CysC) and the reference method chosen by Hoek and Yang, U-Cl(crea-urea) (Figure 3). The analytic method we used for CysC was the same as that used by Yang and Hoek (9,10); however, CysC concentrations measured using the same method in different laboratories may be significantly different (25).

To summarize, eGFR(CysC) seems to have some built-in problems in reflecting GFR, at least in our PD population. Factors other than GFR influencing CysC concentrations could be an explanation. A nonrenal CysC clearance of more than 20 mL/min/1.73 m² has been proposed (23). If that suggestion holds true in dialysis patients, small changes in renal clearance will hardly be detectable, and CysC might not be useful as a marker of GFR in dialysis patients. Together with creatinine, urea, and EDTA, CysC may be dialyzed into the PD fluid to some degree (26,27). Because no validated method was available, we did not measure CysC concentrations in dialysate. Moreover, published eGFR(CysC) values were developed for PD patients without correction for dialysis clearance of CysC. In one study, dialysate clearance was found to be around 0.4 mL/min in anuric adult PD patients (27)—a small and probably insignificant amount compared with the nonrenal clearance of more than 20 mL/min/1.73 m². Studies on CysC kinetics in dialysis patients are greatly needed to elucidate the field.

In pairwise comparison, we found that cP-Cl(EDTA) was 34% higher than U-Cl(EDTA). That difference might partly be caused by extrarenal clearance of ⁵¹Cr-EDTA, which has been proposed but never proved. Another explanation might be methodologic inaccuracy. Plasma clearance is determined by the relation between injected ⁵¹Cr-EDTA and the area under the plasma clearance curve. When GFR becomes low, ⁵¹Cr-EDTA remains in the body for a longer time. The plasma concentration falls slowly, and the area under the plasma clearance curve enlarges. In a non-compartmental model, the approximately mono-exponential plasma concentration curve makes for an extrapolation of the curve from the last blood sample to infinity, and the total area under the curve may be calculated (6). However, in dialysis patients, the slope of the plasma clearance curve is perhaps not exactly mono-exponential. As indicated by detectable ⁵¹Cr-EDTA at the start of visit 2 in the present study, elimination seems to be slower than expected, leading to underestimation of the area under the curve and thus an overestimation of plasma clearance.

Another source of error is that the patients were dialyzed during study days. Even after correction for that nonrenal clearance, dialysis possibly did influence ⁵¹Cr-EDTA plasma concentration to some extent, making the plasma curve determination less reliable. It would therefore be desirable to obtain samples between 5 hours and 24 hours and also later than 24 hours. Moreover, D-Cl(EDTA) might be underestimated, because the abdomen is not always completely drained and sampling time was less than 24 hours. The source of error from dialysate collection might be eliminated by pausing PD through the 24 hours of the investigation. However, the inaccuracy of dialysate collection would then be replaced by the effects of the PD patients no longer being close to steady-state. In our patients, dialysate clearance seemed to vary less than ultrafiltration volume.

To date, only one other study has performed an external validation of eGFR(CysC) in PD patients (11). In that study, ⁵¹Cr-EDTA plasma clearance was used, and the sample drawn after 7 hours was the latest used. Patients were not dialyzed during those 7 hours. Correction for dialysate clearance was therefore unnecessary. Unfortunately, urine was not analyzed for ⁵¹Cr-EDTA. In contrast with our findings, those authors found that eGFR(CysC) was lower than ⁵¹Cr-EDTA plasma clearance, probably because of too-early termination of blood sampling, leading to overestimation of GFR by ⁵¹Cr-EDTA plasma clearance (6,28).

To summarize, cP-Cl(EDTA) probably overestimates GFR, but provides the best reproducibility despite

inaccuracies in the dialysate collection and the determination of the plasma clearance curve.

In contrast with cP-Cl(EDTA), U-Cl(EDTA) is comparable to urinary inulin clearance in advanced renal insufficiency (12), and in the case of complete urine collection, U-Cl(EDTA) is a precise marker of GFR. Bladder catheterization is obviously not acceptable because of discomfort and infection risk for the patient, and U-Cl(EDTA) most likely underestimates true GFR to some extent because of non-perfect bladder emptying. The reliability of urine collection is disputable, and in patients with very low output (<300 mL/24 h, for instance), the inaccuracy could be expected to be unacceptable. Thus, such patients were not included in the present study. We found similar excretion of creatinine at the two visits, supporting the reproducibility of urine collections when patients are in a stable condition. Earlier, urine volume was proposed to be able to substitute for GFR, especially in follow-up studies (29), and urine volume has also recently been reported without clearance measurements (30,31). However, in the present study, urine volume was not correlated with cP-Cl(EDTA), U-Cl(EDTA), or eGFR(CysC). Urine volume might be an acceptable marker of RRF in follow-up studies, but urine output does not seem to be interchangeable with measurements of GFR in PD patients.

To summarize, U-Cl(EDTA) has limitations, especially the risk of incomplete urine collection. This source of error was minimized by training and careful instructions. Although this method was chosen as a reference, it cannot be termed a "gold standard." Unfortunately, no "gold standard" is available for GFR measurements in PD patients, and for the time being, U-Cl(EDTA) is probably the best method in patients able to collect urine.

Urine collection as a source of error was excluded when U-Cl(crea-urea) was compared with U-Cl(EDTA). The comparison showed significant bias, and the methods were thus not interchangeable. However, when GFR is less than 10 mL/min/1.73 m², U-Cl(crea-urea) seemed to overestimate U-Cl(EDTA) by just 12%.

Only 12 patients were included in our study, and generalizability may thus be disputable. The sample, however, seems to be representative of most PD populations, although 2 patients had very high GFRs. However, if demographic variables influence the measurements considerably, that influence should be demonstrated in studies including a larger number of patients. The participants in our study differed from the general dialysis population in being relatively healthy but having no job, which allowed them to spend extra time at the hospital. Moreover, only patients with a urine output above 300 mL/24 h were included.

CONCLUSIONS

If complete urine collection is possible, GFR may be measured using U-Cl(crea-urea) with an overestimation of 12%. The previously proposed eGFR(CysC) equations need further development and validation and are currently not recommendable. Outside of eGFR(CysC), cP-Cl(EDTA) is the most reproducible method, but it is labor-intensive and expensive, and it overestimates GFR to some extent. An easy and reliable method to estimate GFR without 24-hour collections is desirable, but not yet available.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

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