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A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate

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Abstract

Background. In clinical practice, the glomerular filtration rate (GFR) is often estimated from plasma creatinine. Several studies have shown cystatin C (cys C) to be a better parameter for the diagnosis of impaired renal function. No data are available, however, on the performance of cys C in follow-up of patients, compared with creatinine. Also, comparisons of cys C with the Cockcroft and Gault (C&G) formula for estimation of GFR are few.

Methods. Plasma samples were obtained from 93 consecutive patients seen for GFR determination and from 30 patients with diabetes mellitus type 2, of whom 23 were investigated a second time after 2 years. GFR was determined with [^{125}I]iothalamate. Plasma creatinine was determined enzymatically and the creatinine clearance calculated according to C&G. Cys C was measured with a particle-enhanced immunonephelometric method.

Results. GFR correlated with 1/cys C ($r = 0.873$) as well as with C&G ($r = 0.876$). The area under the curve (AUC) of the receiver operating curves (ROCs), a measure of diagnostic accuracy, for cys C (0.931) and C&G (0.938) were equal ($P = 0.815$) and both better than the creatinine AUC (0.848; $P = 0.006$). Bland and Altman analysis showed that the simple formula GFR = –4.32 + 80.35 × 1/cys C, derived from our data, gave more accurate ($P < 0.0001$) and more precise ($P = 0.024$) GFR estimates than obtained with the C&G formula. The day-to-day variation (biological + analytical) for cys C was small (3.1%, SD 2.51%) in diabetic patients. In the follow-up study in diabetic patients, cys C was the parameter which had the best correlation ($r = 0.66$) with changes in GFR.

Conclusions. Cys C shows a high correlation with GFR. With a very simple formula, cys C gives a good estimate of GFR, more accurate and precise than C&G. Because biological variation is low, cys C gives also a good assessment of GFR changes during follow-up. Cys C is the preferred endogenous parameter for GFR.

Keywords: Bland and Altman analysis; Cockcroft and Gault; creatinine; cystatin C; glomerular filtration rate; ROC analysis

Introduction

The determination of the glomerular filtration rate (GFR) is a cumbersome procedure, ideally involving inulin infusion and urine collection under very standardized conditions. In practice, infusion of radioactive substances such as [^{125}I]iothalamate, [^{51}Cr]EDTA or [^{99m}Tc]DPTA is often used. However, this test is performed only when precise information on kidney function is mandatory. In clinical practice, plasma creatinine is measured as an estimate of the GFR, on the assumption that creatinine is completely filtered across the glomerulus and that creatinine production and excretion are constant. The plasma creatinine concentration is then inversely related to the GFR. However, creatinine production depends on muscle mass and is age and sex related. As a result, a wide reference range is found. The Cockcroft and Gault (C&G) formula [1] is used to estimate the creatinine clearance from the plasma creatinine concentration with a correction for age, muscle mass and sex. Other factors that reduce the value of plasma creatinine as a GFR estimate are the substantial tubular excretion of creatinine and the well known sensitivity of the analytical methods, especially the Jaffé method, to interfering substances in the plasma. To apply the C&G formula, plasma creatinine

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needs to be in steady state. The formula is also inaccurate in patients with liver disease, muscle wasting, oedema or extreme adiposity.

Plasma cystatin C (cys C) was proposed some years ago as an alternative endogenous substance, because it has many properties of an ideal marker for GFR [2]. Interest in the use of this protein has grown recently, because immunonephelometric [3] and immunoturbidimetric [4] methods have become available, which allow a rapid and precise routine measurement. A number of cross-sectional studies comparing cys C with plasma creatinine have been published, and in most cases cys C was a more sensitive indicator of mild reductions of renal function than plasma creatinine [5–11]. At least two issues remain, however, which have to be investigated more thoroughly, before cys C can be established as a valuable improvement in the the field of GFR estimation. One, as stressed by Deinum and Derkx [12], is the fact that the value of the plasma creatinine determination can be improved simply by estimation of the GFR with the C&G formula, when a number of simple parameters such as sex, age, weight and height are recorded. Therefore, cys C should be compared with the results obtained with the C&G formula. This comparison has been done so far only in a small number of studies [8,13,14] and the results were not equivocal.

The second issue is the fact that although a large interindividual variation is present for plasma creatinine, the intraindividual variation is small. For cys C, a small interindividual variation but a larger intraindividual variation has been reported [15]. Therefore, the value of plasma creatinine for the follow-up of individual patients might be much better than suggested in the cross-sectional studies and, in contrast, the follow-up results for cys C might be worse.

The objective of the present study was to investigate the usefulness of plasma cys C determination in a cross-sectional analysis comparing plasma cys C with plasma creatinine, C&G estimated creatinine clearance and GFR, and also during longitudinal follow-up of patients with type 2 diabetes mellitus. Obesity is often present in diabetic patients, which may interfere with the accuracy of the C&G formula.

Subjects and methods

Patients

The study was performed in a cohort of 93 patients, who were seen at the Nephrology Department of the Academic Medical Center in Amsterdam for a determination of GFR for suspected or established renal dysfunction. Height, weight and age were recorded. In addition, a group of 30 patients with diabetes mellitus type 2, equally distributed over the normo-, micro- and macroalbuminuric range, was investigated, whose plasma samples were available, frozen at –80°C. The indications for performing a GFR and further patient characteristics are summarized in Table 1.

The diabetic patients were the subjects of studies regarding the influence of cimetidine administration on GFR estimation, as described by Kemperman *et al*. [16]. For 23 of these patients, these studies were repeated 2 years later, allowing an estimation of the parameters for follow-up. Thus, overall, 146 plasma samples were used for the evaluation of cys C.

From the diabetic patients investigated in those studies, an additional 42 samples were available, taken during cimetidine administration, which were used for a between-day comparison of the cys C level.

Analytical methods

The gold standard for GFR consisted of a continuous infusion of ^{125}I-labelled iothalamate and ^{131}I-labelled hippuran, enabling a simultaneous determination of GFR and effective overall plasma flow [17,18]. With this method, GFR is calculated as the mean urinary clearance of [^{125}I]iothalamate of two 2-h periods after a 2-h equilibration period. Corrections were made for incomplete urinary collections and fluctuations in plasma concentrations, as described previously [17–20]. The creatinine clearance was calculated with the C&G formula [1]:

$$[140 - \text{age (years)}] \times \text{body weight (kg)}/[0.815 \times \text{plasma creatinine } (\mu\text{mol/l})]$$

For women, the correction factor of 0.85 was used.

All clearances were expressed as ml/min/1.73 m^2 after correction for body surface area (BSA) according to the DuBois–DuBois formula [21]:

$$\text{BSA } (\text{m}^2) = 0.007184 \times \text{height } (\text{cm})^{0.725} \times \text{weight } (\text{kg})^{0.425}.$$

Table 1. Patient characteristics

Indication for GFR	No. of subjects	M/F	Age (years)	Weight (kg)	GFR (ml/min/1.73 m^2)
SLE and systemic vasculitis	26	6/20	35 (19–73)	68.2 (42–95.7)	69.3 (12.4–142.3)
Various glomerulopathies	20	12/8	40 (19–64)	75.5 (51.6–111)	70.8 (32.1–149.1)
Work-up living kidney donor	19	11/8	51 (27–77)	78 (45–105)	101.7 (67.5–149.0)
Follow-up after kidney transplantation	4	1/3	54 (45–57)	71.6 (63.1–90)	34.2 (12.2–58.6)
Prior to lung transplantation	5	3/2	58 (53–67)	69 (55–97)	103.6 (78.1–136.1)
Hypertension	5	2/3	45 (35–60)	82.4 (55–100)	83.0 (45.4–119.9)
Renal failure	4	1/3	56 (24–66)	76 (45–96.5)	36.7 (14.0–63.3)
Interstitial nephritis	3	1/2	29 (27–35)	54 (44–99)	50.6 (33.5–80.6)
Miscellaneous	7	4/3	20 (11–73)	60 (25–93)	51.4 (17.0–119.7)
Diabetes mellitus type 2	30	18/12	55 (30–70)	80 (53–115)	89.5 (37.0–157.0)

Data for age, weight and GFR are given as the median, and the total range is given in parentheses.

The creatinine concentration in plasma was measured with an enzymatic PAP+ (phenol/4-aminoantipyrine) assay on a Hitachi 747 analyser (Roche Diagnostics, Mannheim, Germany). The upper limit of the creatinine reference range was 110 μmol/l for males and 95 μmol/l for females.

Cys C was measured in heparinized plasma samples with the N Latex Cystatin C test kit, a particle-enhanced immunonephelometric method, on a BN ProSpec analyser (Dade Behring, Leusden, The Netherlands). A within-run CV of 1.52% was found with a plasma pool containing 1.05 mg/l cys C. The between-run CV was tentatively established with the N Cystatin C control (1.40 mg/l cys C) at 1.67% ($n = 8$). The reference range used for cys C was 0.5–0.96 mg/l, as recently established by Finney *et al.* [3] with the same reagents on a BN II analyser.

We tested if the cys C result can be used to calculate a GFR, in the same way as creatinine is used to calculate a C&G clearance, and what accuracy and precision are then achieved. First, the 93 consecutive GFR determinations were sorted in order of their GFR result; all uneven numbers were referred to the reference group for calculation of the optimal regression equation, and with the even numbers a test group was formed. In the diabetic patients, the results from the first year were used to derive the regression equation, and cys C results obtained in the samples from 2 years later were used to test the GFR estimation. The slope and intercept for the equation derived in the diabetic reference group were within the confidence interval of the other equation. Therefore, both reference groups were combined for the analysis presented here.

Statistical methods

For the calculations, SPSS for Windows, release 9.0.1 was used. For comparisons between groups, Pearson's correlation was used. The significance of differences between means was calculated with the *t*-test; for comparison of SDs the *F*-test was used. Equations giving the best fit with the data were calculated with Table Curve software from Jardell Scientific. The sensitivity and specificity of an assay depend on the cut-off which is chosen. Receiver operating characteristic (ROC) curves give a graphical display of the performance of a test. Test sensitivity is plotted *vs* 1 – specificity, with each point of the curve representing a different cut-off level. The area under the curve (AUC) describes the test's overall performance and is used to compare different tests. For the ROC curves, AUCs were calculated and compared using Medcalc software. This program applies the Hanley and McNeil method [22] for the non-parametric estimation of the AUC.

The Medcalc software was also used for a Bland and Altman analysis [23] of the GFR estimates, compared with the measured GFR. Accuracy and precision are determined in this type of analysis by relating the difference between estimated and measured GFR to the average of measured and estimated GFR in each patient. Whether a systematic increase or decrease of the difference is present with increasing GFR is checked by inspection of the graph. The limits of agreement are given by the mean ± 1.96 SD, containing 95% of the values. The mean difference is a measure of accuracy; the SD is a measure of precision.

Results

The median age of the patients was 50 years, range 11–77 years, with equal numbers of males and females. GFR results ranged from 12.3 to 157 ml/min/1.73 m^2, median 81 ml/min/1.73 m^2, and were normally distributed over that range. Twenty-nine results were < 50 ml/min/1.73 m^2, 36 were between 50 and 75, 42 were between 75 and 100, 28 were between 100 and 125, and 11 were higher than 125 ml/min/1.73 m^2. We measured cys C levels from 0.53 to 5.09 mg/l, median 1.01 mg/l, and the creatinine concentrations found ranged from 38 to 335 μmol/l, with a median of 73 μmol/l. The results for the plasma markers for GFR, differentiated for these different levels of GFR, are given in Table 2. A graphical presentation of the results is given in Figure 1. The cys C results started to become abnormal at a GFR level of ~80–90 ml/min/1.73 m^2; the GFR did reach the 60–70 ml/min/1.73 m^2 range before creatinine exceeded the upper reference limit. Overall, the correlation between 1/cys C and GFR was highly significant [$r = 0.873$; 95% confidence interval (CI) 0.828–0.907; $P < 0.0001$] and significantly better ($P = 0.038$) than between 1/creatinine and GFR ($r = 0.800$; 95% CI 0.733–0.852; $P < 0.0001$). The correlation coefficient observed for the relationship between the C&G creatinine clearance and the GFR was $r = 0.876$ (95% CI 0.832–0.909).

In order to determine the diagnostic accuracy of cys C for the detection of an abnormal GFR compared with the other available markers, ROC plots were constructed. The GFR determined with [^{125}I] iothalamate was used as the gold standard. Because different cut-off

Table 2. Plasma markers for GFR in relation to renal insufficiency

GFR range (ml/min/1.73 m^2)	*n*	Cystatin C (mg/l)	Creatinine (μmol/l)	C&G (ml/min/1.73 m^2)
< 50	29	1.89 (1.69–2.54)	137 (113–187)	52 (41–58.5)
50–75	36	1.21 (1.10–1.33)	91 (74.9–99.6)	74.8 (68.4–82.7)
75–100	42	0.89 (0.84–0.93)	69.5 (63.4–74.5)	105.0 (93.9–109.9)
100–125	28	0.77 (0.68–0.81)	61 (55.8–68)	128.3 (117.6–138.4)
> 125	11	0.67 (0.55–0.90)	56 (40.8–65.9)	148.9 (133.2–175.9)

The results are expressed as median and interquartile range. Although the overall correlation of C&G with GFR is excellent, the results obtained for C&G in comparison with the GFR ranges clearly illustrate that a strong positive bias exists. This overestimation is due mainly to tubular excretion of creatinine, which can be inhibited by cimetidine [16].

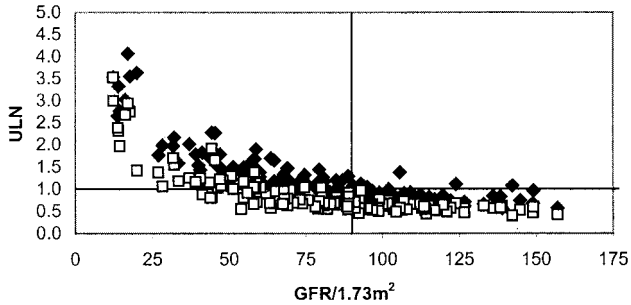

Fig. 1. Relationships of cys C (diamonds) and creatinine (squares) to GFR for all 146 plasma samples investigated. Cys C and creatinine are both given as a ratio relative to their respective upper limits of the reference range (ULN). For males and females, their gender-specific creatinine ULN was used. The lower reference limit for GFR of 90 ml/min/1.73 m^2 is also indicated in the figure.

levels can be chosen to define the borderline between normal and abnormal glomerular filtration, we calculated various ROC plots, with GFR limits at 90, 80, 70 and 60 ml/min/1.73 m^2. At these GFR cut-offs, cys C showed, with the upper reference limit as cut-off, a sensitivity of 76.9, 91.4, 94.8 and 97.7% and a specificity of 89.1, 84.2, 76.1 and 67.0%, respectively.

Also the C&G data and the creatinine data were expressed relative to these cut-off values. The AUCs of the ROC curves obtained at the different GFR cut-offs are presented in Table 3.

Both C&G and cys C always gave higher AUCs and thus better diagnostic accuracy than creatinine. The difference was always significant ($P < 0.001$) except for the GFR cut-off at 60 ml/min/1.73 m^2, where the difference did not reach significance ($P = 0.121$). The cys C curve always showed equal accuracy compared with C&G.

Estimation of GFR

In the C&G formula, the plasma creatinine concentration, together with data on body weight, age, height and sex, is used to calculate an estimate for GFR/1.73 m^2. We tested if the cys C result can be used in the same way to calculate a GFR and what accuracy and precision are then achieved.

The resulting equation was

GFR/1.73 $m^2 = -4.32 +$ 80.35/cys C, with $r^2 = 0.872$.

The 95% CI for the intercept was –12.46 to 3.81, and for the slope it was 72.78–87.93.

The difference, calculated according to Bland and Altman [23] between the GFRcys calculated with this equation in the test group of 69 samples and the GFR determined with the reference method was –2.4 ± 12.09 (SD) ml/min. The difference between C&G and GFR was 15.9 ± 15.41 (SD) ml/min (Figure 2). Not only was the mean difference significantly lower for the GFRcys ($P < 0.0001$), but the SD was also significantly smaller ($P = 0.024$).

The accuracy, but not the precision, of the C&G calculation is greatly improved when the plasma creatinine is measured after cimetidine administration [16]. This $C\&G_{CIM}$ result in 23 patients with type 2 diabetes gave a difference in the GFR of 0.13 ± 17.99 ml/min.

Table 3. ROC curve comparisons for C&G, cystatin C and creatinine

GFR cut off level	Parameter	AUC (95% CI)	*P*-value[a]
≥60 ml/min/1.73 m^2	C&G	0.955 (0.907–0.982)	0.627
	Cystatin C	0.963 (0.919–0.987)	–
	Creatinine	0.924 (0.869–0.962)	0.121
≥70 ml/min/1.73 m^2	C&G	0.953 (0.905–0.981)	0.787
	Cystatin C	0.958 (0.911–0.984)	–
	Creatinine	0.890 (0.828–0.936)	0.008
≥80 ml/min/1.73 m^2	C&G	0.951 (0.902–0.980)	0.679
	Cystatin C	0.959 (0.912–0.985)	–
	Creatinine	0.877 (0.812–0.925)	0.001
≥90 ml/min/1.73 m^2	C&G	0.946 (0.896–0.976)	0.552
	Cystatin C	0.932 (0.878–0.967)	–
	Creatinine	0.877 (0.812–0.925)	0.028

[a] *P*-values are given relative to the cystatin C results.

Biological variation

Plasma samples taken for a study of the effect of cimetidine administration on the C&G creatinine clearance were available for analysis. Consequently, cys C could be determined in paired samples, taken 2–3 days apart at the same time of day. For this comparison, 42 sample pairs were available. The day-to-day CV ranged from 0 to 9.8%, and was 3.1 ± 2.51%.

GFR change in time

In 23 type 2 diabetes patients, changes in GFRcys calculated from cys C levels were compared with changes in GFR measured after 2 years. Because in these patients GFR was also measured after cimetidine administration, almost always two plasma samples were available. When this was the case, the first cys C result was used for the calculations. The changes observed in the individual samples were expressed as a percentage of the start value.

Urinary albumin excretion was < 3 mg/mmol creatinine in seven patients, between 3 and 30 mg/mmol creatinine in eight patients and > 30 mg/mmol creatinine in eight patients.

The GFR changes in the patients with normoalbuminuria, with microalbuminuria and with macroalbuminuria are given in Table 4. Overall, for GFRcys as well as for C&G and $C\&G_{CIM}$, the changes correlated significantly with changes in GFR. The highest correlation was found for GFRcys, but the difference with the other correlations did not reach statistical significance.

Discussion

The results of this study show that cys C is the most useful endogenous indicator of GFR for diagnosis and

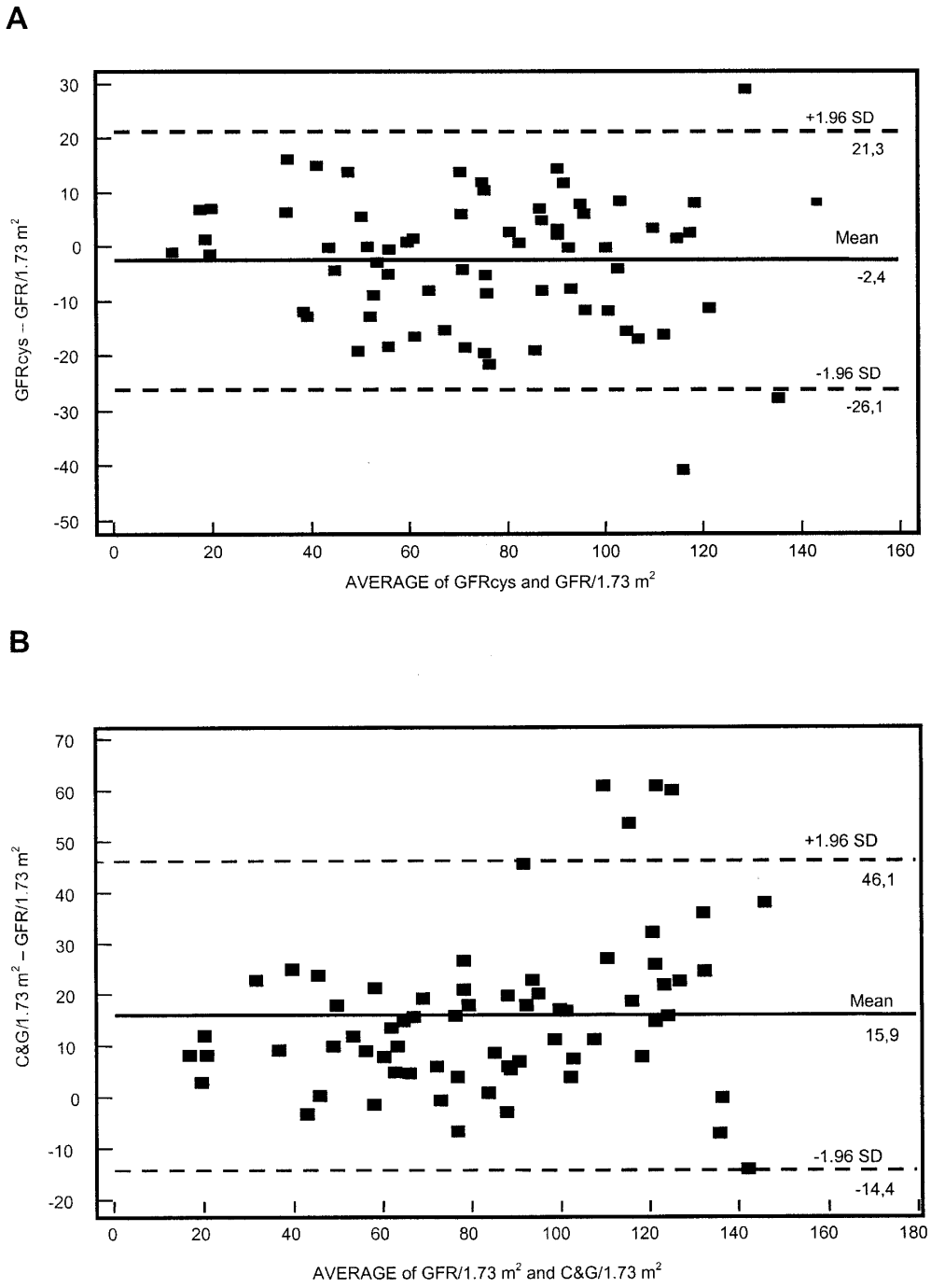

Fig. 2. Bland and Altman plot for differences between estimated GFR and measured GFR. On the *x*-axis, the average GFR is given and on the *y*-axis the difference in ml/min between the estimated GFR, derived from the cys C formula (**A**) or the C&G formula (**B**) is given. The mean difference (solid lines) and the 1.96 SD limits (dotted lines) are also plotted.

follow-up. We compared results of cys C and plasma creatinine with the GFR measured with the gold standard, the continuous infusion [^{125}I]iothalamate method (Figure 1). Cys C results started to become abnormal close to the cut-off level of the reference range for GFR of 90 ml/min/1.73 m^2. At this level, plasma creatinine values were still within the reference range. Our results are in close agreement with the findings of Coll *et al.* [9], who started to observe greater than normal cys C levels when GFR was ~88 ml/min/1.73 m^2. Their serum creatinine levels, on the other hand, became abnormal when GFR was ~75 ml/min/1.73 m^2. The correlation with GFR of the reciprocal values of cys C and plasma creatinine is better in our study than in the study of Coll *et al.* [9], but is still in the range observed in the literature [5,11]. The correlation for 1/cys C is better than for 1/creatinine and is similar to the correlation between C&G and GFR. These high

Table 4. Changes in GFR and plasma markers for GFR after 2 years

	No albuminuria ($n=7$)	Microalbuminuria ($n=8$)	Macroalbuminuria ($n=8$)	R (95% CI)
Median GFR at the start (ml/min/1.73 m^2)	104.0 (92.2–112.2)	75.5 (65.5–107.0)	77.0 (51.5–104.5)	
ΔGFR	−4.49 (−6.69 to 7.35)	−2.12 (−7.73 to −0.11)	−25.19 (−36.84 to −13.52)	–
ΔGFRcys	5.05 (−4.63 to 15.85)	−10.62 (−25.43 to −0.72)	−17.72 (−24.50 to −11.00)	0.6628 (0.3448–0.8443)
ΔC&G	−1.18 (−4.42 to 8.51)	−4.92 (−7.21 to 2.91)	−11.57 (−27.23 to −6.81)	0.5583 (0.1898–0.7889)
ΔC&G$_{CIM}$	−5.41 (−17.10 to 11.07)	−4.70 (−7.54 to 7.36)	−16.85 (−24.44 to −1.59)	0.5521 (0.1812–0.7856)

Results for the changes observed after 2 years are given as median and interquartile range, as a percentage of the start value. The correlation with changes in GFR is given and is significant at the 0.001 level for GFRcys and at the 0.01 level in the other cases.

correlations are of course facilitated by the regular distribution of GFR values in our study population, but the analytical precision of the cys C method on the ProSpec analyser and the use of an enzymatic creatinine assay may also have contributed.

For our study, we have used, together with recently taken samples, some samples from studies in diabetic patients dating from 1996 and 1998 and kept at –80°C. The results of cys C and creatinine determinations performed recently have thus been compared with GFR determinations from 2–4 years before. For cys C, this storage period is much longer than the 6-month period in which no deterioration was observed in other studies [3,5,6]. Still, the results obtained in these samples do not give the slightest indication that any degradation of cys C might have occurred. Therefore, we presume that plasma samples for cys C can safely be stored at –80°C for at least 4 years.

The diagnostic accuracy for cys C and C&G, as shown by the AUCs of their ROC curves (Table 3), is comparable, in contrast to plasma creatinine, which has lower accuracy. The definition of the GFR reference limit, below which glomerular function is impaired, influences the diagnostic accuracy of the methods under investigation. At a lower GFR limit, a somewhat higher sensitivity is found for cys C at a given cut-off, but at the cost of a lower specificity. In addition, the lower the GFR limit chosen, the more the ROC curves for C&G, cys C and plasma creatinine approach each other. A GFR limit below 80 ml/min/1.73 m^2 hardly influences the diagnostic accuracy of cys C (almost equal AUCs), but improves the diagnostic accuracy of plasma creatinine. The cut-off values for cys C for optimal sensitivity and specificity are at or near the upper reference range. However, for creatinine, the optimal cut-off values in the curve overlap completely with the reference range, which underlines once more the fact that plasma creatinine has little value for the diagnosis of minor renal impairment, but that it can be useful only in the follow-up of patients in time. The good diagnostic accuracy of the C&G clearance is observed only at cut-off levels much higher than the upper reference limit of GFR. This well known discrepancy between C&G and GFR is mainly the consequence of the tubular excretion of creatinine. It can be avoided by measuring the plasma creatinine for the C&G calculation after administration of cimetidine and inhibition of this tubular excretion route [16].

Three studies are available in the literature comparing cys C, creatinine and C&G data [8,13,14]. Risch *et al*. [13] studied renal transplant patients and found a better correlation with GFR for cys C than for C&G. Using a GFR cut-off of 60 ml/min, the AUC of the ROC curve for cys C was higher than for creatinine in their study. Oddoze *et al*. [14] reported in diabetic patients with early renal impairment a higher correlation for creatinine with GFR than for cys C or C&G. At a GFR cut-off of 80 ml/min, the AUC for cys C was 0.780, much lower than found by us and also significantly lower than the mean AUC in a recent meta-analysis [11]. The study by Chantrel *et al*. [8] was performed, like our study, in patients with various pathologies. They did not find any significant difference between the AUCs of cys C, C&G or creatinine at a GFR cut-off of 90 ml/min. All studies used a Jaffé creatinine method, and the latter two used the same immunonephelometric cys C method as we did. Our results for the cys C correlation with GFR correspond to the higher mean value for the immunonephelometric method reported in the meta-analysis [11], and the creatinine correlation is even higher than the mean of reported results. According to the meta-analysis [11], a lower ROC-plot AUC should be found for creatinine compared with cys C, as we did. The very diverse outcomes of the studies by Chantrel [8], Oddoze [14] and Risch [13] make a comparison difficult.

This study shows that, in addition to the information cys C gives on the presence or absence of even mild renal dysfunction, its level can also be used in a simple formula to give a significantly more accurate and precise quantitative estimate of GFR than obtained by C&G. It does this irrespective of sex, age, weight and height, all those additional data which are necessary for a C&G calculation. The Bland and Altman analysis presented in Figure 2 shows that this simple cys C formula is superior to C&G over the total range of GFR which was investigated, and also in the range of minor renal impairment. These findings are all in favour of the use of cys C for estimating GFR instead of the C&G formula. The utmost accuracy for the estimation of the GFR so far could only be achieved when a C&G$_{CIM}$ was calculated from the plasma creatinine under cimetidine administration. The calculation from

cys C equals even that accuracy in the group of diabetic patients and is of course much simpler.

The slope and intercept of the formula, as found by us, may very well be method dependent. Not only are differences in correlation with GFR observed between the immunonephelometric method and other methods [11], but differences in standardization between methods also result in different reference ranges and different test outcomes.

Although cys C is produced endogenously generally at a constant rate, one exception has been reported. Higher serum cys C levels and underestimation of GFR have been found in children on immunosuppressive therapy after renal transplantation [24]. *In vivo* and *in vitro* evidence was presented for the influence of glucocorticoid therapy on the cys C production rate and consequently on its serum level [25,26]. Risch *et al*. [26] reported that the extra cys C increase depended on the creatinine clearance level and varied from 0.20 mg/l at 80 ml/min/1.73 m^2 up to 1.85 mg/l at 20 ml/min/1.73 m^2 on low-dose glucocorticoids compared with controls. The value of these findings is difficult to assess, because cys C was only compared with C&G as an estimate of the GFR. Prednisone therapy was shown earlier to be associated with an increase in GFR and urinary creatinine excretion rate, but also with an increase in plasma creatinine concentration [27]. The effects on cys C concentration described by Risch *et al*. would in our GFR estimation result in a 14.1 ml/min lower outcome for GFR at 80 ml/min/1.73 m^2 and a 8.1 ml/min lower outcome at 20 ml/min/1.73 m^2. Even though this underestimation is within the variation range observed in our patients, a further evaluation of the use of cys C for GFR estimation compared with C&G estimation is warranted in renal transplant patients on glucocorticoid therapy.

Another point of concern about the applicability of cys C for the assessment of GFR is the large intraindividual variation observed for this protein. In combination with a much higher variability than achieved for creatinine, this results in a critical difference between two consecutive observations much larger than for plasma creatinine. This observation was made in healthy volunteers [15]. Our duplicate results in diabetic patients with generally only minor impairment of their GFR show a mean day-to-day CV of 3%, in sharp contrast to the 13% biological variation in healthy volunteers. Therefore, it seems likely that confounding factors, which may cause a significant biological variation in healthy individuals, play only a minor role in patients. These findings are supported by the data from the follow-up of the renal function of diabetic patients. The best correlation with changes in GFR is found for GFRcys, a result which contradicts the existence of more significant biological variation for cys C than for creatinine.

In conclusion, all data presented here support the value of cys C as the endogenous parameter for estimation of GFR. The high correlation of cys C with GFR permitted the calculation of a reliable formula for estimation of GFR from cys C data. Although analysis of the data with ROC curves did not show any difference in diagnostic accuracy between cys C and C&G results, the Bland and Altman analysis showed that the GFR estimates from cys C had better accuracy and precision. In a group of type 2 diabetic patients, cys C was also the best parameter for follow-up of GFR changes.

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