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Original Article

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A comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two

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Abstract

Background. Current recommendations (KDIGO and NKF-K/DOQI) are that patients with chronic kidney diseases (CKD) should be classified in stages 1–5 based on GFR. A serum creatinine-based prediction equation (abbreviated MDRD formula) can be used to estimate GFR (eGFR). Cystatin C has been proposed as an alternative filtration marker to creatinine. We present validation of currently used formulae for eGFR based upon s-creatinine and s-cystatin C and we compare two different methods for the determination of cystatin C.

Methods. S-cystatin C and s-creatinine were measured in 644 patients referred for determination of GFR by plasma clearance of iohexol during the period 1 June 2004 to 31 December 2005. S-cystatin C was determined by turbidimetry using two different reagents (DAKO A/S and Gentian A/S). The 644 patients were divided into two groups. Group 1 was used to calculate own eGFR-formulae based on s-cystatin C (Orebro-cyst). Group 2 was used to validate the formulae. Three creatinine-based equations (Cockcroft–Gault, MDRD and Jelliffe) and seven cystatin C-based (Larsson, Hoek, Filler, leBricon, Grubb and Orebro-cyst DAKO, Gentian) were evaluated. Evaluation was done according to the recommendations by K/DOQI.

Results. In the test sample (group 2) mean GFR (iohexol clearance) was 50.4 ml/min/1.73 m^2 (range 12–150)-mean s-cystatin C (DAKO AS) was 1.63 mg/l and mean s-cystatin C (Gentian AS) 1.92 mg/l. The s-cystatin C concentrations obtained by the Gentian method were approximately 10% lower than the DAKO method within the normal GFR range but were approximately 40% higher within the low GFR range. Bias for the creatinine-based equations was in the range –0.9 to 5.9 ml/min/1.73 m^2 and for the cystatin C-based equations in range –2.4 to 7.9 ml/min/1.73 m^2. Accuracy within 30% ranged from 68.6 to 80.4% and 54.0 to 82.9%, respectively. By combining both, an accuracy within 30% for 87.0% could be reached (MDRD/cystatin C by Gentian). Overall the patients were correctly classified for the different stages of CKD in 62.1–64.0% for the creatinine-based equations, 61.5–72.0% for the cystatin C-based equations and 70.2–73.9% for the combination.

Conclusion. Estimating GFR using formulae based on s-creatinine or s-cystatin C alone was equally accurate according to the NKF K/DOQI guidelines. A formula that combines both provided a greater accuracy. If Cystatin C, which is clearly more expensive, is used, the choice of the cystatin C determination method and an adjusted prediction equation is essential. Use of the IDMS-traceble MDRD seems to yield the best cost–benefit ratio for routine practice.

Keywords: creatinine; cystatin C; GFR; MDRD; prediction equations

Introduction

The Kidney Disease Improving Global Outcomes (KDIGO) as well as the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (K/DOQI) recommends that patients with chronic kidney disease (CKD) or renal transplant recipients should be classified into stages based upon their glomerular filtration rate [1]. A serum creatinine-based prediction equation has been proposed for estimating the glomerular filtration rate (eGFR) [2] and the abbreviated Modification of Diet in Renal Disease formula (MDRD) is the current recommendation [3]. Sources of error for the determination of GFR from s-creatinine are, besides the problems of standardising the analytical method, variation in production rate and tubular secretion [4,5]. Cystatin C, a small, non-glycosylated 13 kDa basic protein, has been proposed as an alternative filtration marker to creatinine [6,7]. Cystatin C is produced at a constant rate with renal elimination being solely by glomerular filtration. The extra renal elimination has been measured in rats and calculated in humans [8,9]. Several formulae for estimating GFR based upon s-cystatin C determination have been proposed. Since there is no international standard for cystatin C these GFR estimates vary with the analytical method and the formula the local laboratory uses to calculate the

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Table 1. Patient characteristics of the two study groups

	Sample for calculating the formulae (Orebro-cyst), mean (SD)	Sample for testing the formulae, mean (SD)	*P*
N	322	322	
Sex (m/f)	186/136	189/133	
Age	56.2 (15.7)	56.5 (15.4)	0.790
GFR (iohexol clearance), ml/min/1.73 m^2	51.7 (30.2)	50.4 (28.1)	0.572
S-creatinine, μmol/l	150.9 (87.5)	153.8 (92.9)	0.678
S-cystatin C (Gentian), mg/l	1.94 (0.94)	1.92 (0.93)	0.860
S-cystatin C (DAKO), mg/l	1.65 (0.7)	1.63(0.7)	0.749
Height	170.3 (9.4)	170.9 (9.4)	0.476
Weight	77.9 (17.2)	76.0 (15.9)	0.139
BSA, m^2	1.89 (0.22)	1.87 (0.21)	0.396
LBM, kg	64.6 (10.1)	65.1 (10.2)	0.518

GFR from the analysis result. Gold standard measurement of GFR employs inulin as a filtration marker [10]. Other exogenously administered substances which have been successfully used as filtration markers include ^{125}I-iothalamate, ^{51}Cr-EDTA and iohexol [11–13]. These methods can be used to validate the various eGFR formulae based upon creatinine and cystatin C, but are not practical for use in daily clinical care.

In this article we present validation of currently used formulae for estimating GFR based upon s-creatinine and s-cystatin C. In addition we compare two different methods for the determination of cystatin C. Part of this work has been presented in EDTA 2005 and ASN 2005 [14,15].

Subjects and methods

Patients

S-cystatin C and s-creatinine were measured in 644 patients (14–91 years) referred to the University Hospital of Orebro for determination of GFR by plasma clearance of iohexol during the period 1 June 2004 to 31 December 2005. Height and weight were noted. The patients were randomly divided into two samples. The patients were listed in order of date of examination and in the case of more than one patient per day by date of birth. Every other patient was placed in group 1 and every other in group 2. In Table 1, the characteristics of the two groups are shown.

Formulae

Group 1 was used for calculating the eGFR formulae from s-cystatin C (Orebro-cyst) and Group 2 was used for testing all the formulae.

The Orebro-cystatin C formula was determined using the calculated production rate (Cys-pr) and extra renal clearance (CL-nr) of cystatin C; GFR = Cys-pr/s-cystatin C – CL-nr [8]. If Cys-pr and CL-nr are constant for differing GFR levels the relationship 1/s-Cys to GFR is linear. Thus Cys-pr and CL-nr can be calculated from the regression $1/\text{s-Cys} = A * \text{GFR} + B$, where Cys-pr = $1/A$ and CL-nr = B/A.

The creatinine-based formulae were Cockcroft and Gault [16], Jelliffe [17], and the re-expressed MDRD-formula from 2005, MDRD with a standardised s-creatinine assay [18]. In addition combinations of formulae were assessed, the mean of MDRD and Orebro-cyst DAKO and the mean of MDRD and Orebro-cyst Gentian.

A summary of the GFR estimating formulae based on s-cystatin C is shown in Table 2 [8,19–23].

Cystatin C concentrations, determined using reagents from DAKO, were used for the eGFR formulae of Larsson, Grubb and Orebro-cyst DAKO. Since the formulae by Hoek and le Bricon were based on the nephelometric determination of cystatin C using reagents from Dade-Behring, the cystatin C concentrations obtained with the Gentian method were used for these formulae. Agreement between the Gentian and Dade-Behring methods has been shown ($R^2 = 0.9945$) [24].

Laboratory methods

GFR was determined by measuring the plasma clearance of iohexol [25,26]. Serum iohexol concentrations were determined using a HPLC method. The total (intra- and interassay) coefficient of variation of the method was 2.6% (concentration 65 mg/l). The single sample technique was used. A serum sample at $t = 0$ h was drawn and 5 ml of iohexol 647 mg/ml was injected. The time for drawing the second serum sample was based upon an estimate of GFR using the serum creatinine concentration. The times used were between 3.5 and 24 h.

S-cystatin C and s-creatinine were determined on the same serum samples as iohexol using a Hitachi 911 autoanalyser. Creatinine (enzymatic method) was determined using reagents from Roche Diagnostics (Mannheim, Germany) and the calibrator was IDMS standardised. The total coefficient of variation for creatinine was 2.2% (concentration 48 μmol/l). Cystatin C was determined by turbidimetry using reagents from DAKO (DAKO A/S, Glostrup, Denmark) and Gentian (Gentian AS, Moss, Norway). The total coefficient of variation for cystatin C was 4.0% (concentration 0.8 mg/l) and 4.6% (concentration 1.2 mg/l) for Gentian and DAKO, respectively.

Methods for evaluating the equations

The prediction equations were evaluated according to recommendations in the NKF K/DOQI guidelines (2).

Table 2. GFR estimating formulae based upon s-cystatin C

Reference	Proposed formula	Unit	Number of patients	Patient characteristics
Larsson *et al.* [19]	GFR = 99.43 ∗ s-cystatin $C^{-1.5837}$	ml/min	100	Adults referred for measurement of iohexol clearance
Hoek *et al.* [20]	GFR = 80.35/s-cystatin C–4.32	ml/min/1.73 m^2	123	Adults with renal disease
Filler *et al.* [23]	GFR = 91.62 ∗ s-cystatin $C^{-1.123}$	ml/min/1.73 m^2	536	Children
Grubb *et al.* [21]	GFR = 84.69 ∗ s-cystatin $C^{-1.680}$ ∗ 1.384 (age < 14 years)	ml/min/1.73 m^2	536	Children and adults referred for determination of GFR by iohexol clearance
Le Bricon [22]	GFR = 78/s-cystatin C+4	ml/min/1.73 m^2	25	Adults with renal transplants
Orebro-cyst (DAKO) [8]	GFR = 119/S-cystatin C—33	ml/min/1.73 m^2	393	Adults referred for determination of GFR by iohexol clearance
Orebro-cyst (Gentian) [8]	GFR = 100/S-cystatin C—14	ml/min/1.73 m^2	393	Adults referred for determination of GFR by iohexol clearance

Evaluation of bias, precision, accuracy and correlation (R) were made.

Paired samples t-test was used to compare the different eGFR formulae with clearance of iohexol (reference). The mean difference (bias) between the paired observations is given with SD (precision) and P-values. Relative differences were calculated as percentage difference from the measured GFR. Accuracy is the proportion of estimates within 30 or 50% of the reference GFR. Weighted Kappa statistics were used to evaluate the agreement between stages classification from CL iohexol and from the other eGFR methods. The K value can be interpreted as follows: Poor (<0.20), Fair (0.21–0.40), Moderate (0.41–0.60), Good (0.61–0.80) and Very good (0.81–1.0).

Statistical analyses were performed using MedCalc for Windows, version 8.1.0.0 (MedCalc Software, Mariakerke, Belgium).

Results

The two different turbidimetric methods used here for determining cystatin C gave different results, which can be explained by differences in standardisation of the methods. We found that the concentrations obtained by the Gentian method were approximately 10% lower than the DAKO method within the normal GFR range but were approximately 40% higher within the low GFR range (Figure 1). The linear regression between 1/s-cystatin C and GFR showed a better correlation with the Gentian method compared with the DAKO method ($R = 0.9322$ and 0.8350, respectively). The eGFR formulae obtained from measurement of s-cystatin C differed according to the method used: GFR = 119/S-cystatin C—33 ml/min/1.73 m^2 (DAKO) and 100/S-Cystatin C—14 ml/min/1.73 m^2 (Gentian). The calculated estimations of creatinine clearance, GFR-creatinine, GFR-cystatin C and the combined GFR-creatinine/cystatin C compared with the reference GFR method (clearance of iohexol) as regards bias, precision and accuracy are presented in Table 3.

As shown in Table 3 all eGFRs except Jelliffe, the MDRD equation, GFR-cystatin C by Hoek and the mean MDRD/Orebro-cyst DAKO were positively biased, i.e. the calculated eGFR overestimated the measured GFR. Best accuracy within 30% was reached with the formulae using cystatin C analysed by the Gentian method. The accuracy within 30% for the Orebro-cyst Gentian formulae was 82% and for the mean MDRD/cystatin C Gentian formulae the corresponding value was 87.0%. The percentage difference from the measured GFR is shown as relative difference. Mean 20.9% in relative difference was found for the MDRD formula, 26.9% for Cockcroft–Gault and 17.2–28.3% for the different cystatin C-based formulae. The linear correlations (R) varied from 0.77 to 0.95.

Table 4 shows how many patients were correctly classified for the different stages of CKD, according to GFR estimating equations based upon s-creatinine and s-cystatin C. Overall a correct classification was achieved in 62.1 to 64.0% of the patients with the creatinine-based formulae and 61.5 to 72.0% with the cystatin C-based formulae. The best result (73.9%) was achieved with the mean MDRD/cystatin C Gentian formula.

Discussion

In this study we have compared different formulae based upon creatinine and cystatin C for estimating GFR. We have shown that the different published formulae give rise to different GFR estimates from the same concentrations of these analytes. The formulae based on the mean of the eGFRs from s-creatinine and s-cystatin C performed best, 73.9% of the patients being correctly classified in CKD stages one to five by the mean MDRD/cyst C Gentian formula. The accuracy of this formula within 30% was also the highest at 87.0%.

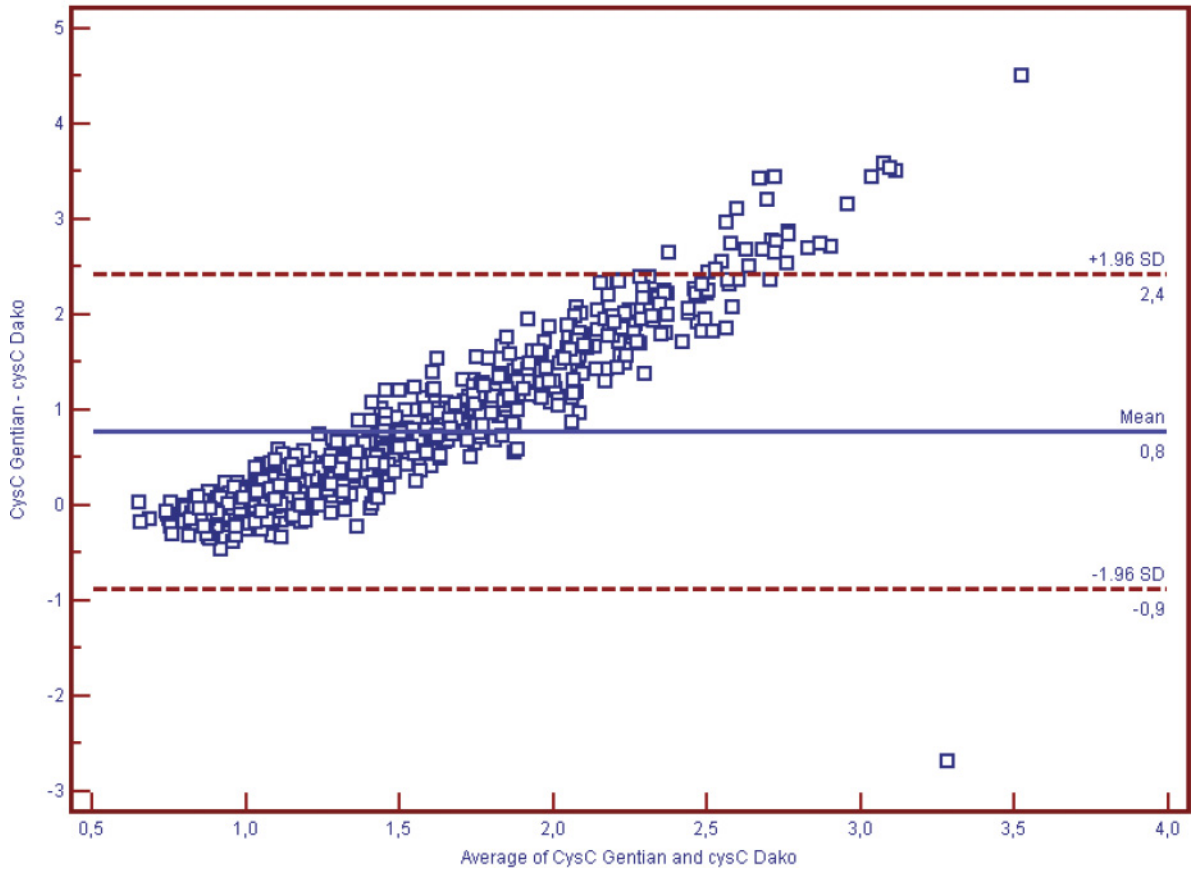

Fig. 1. Altman–Bland plot showing a comparison between the two methods of determining s-cystatin C (Gentian relative to DAKO). $N = 644$.

Table 3. Results of calculated estimations of creatinine clearance and GFR, bias, precision and accuracy related to the reference GFR method (clearance of iohexol) of s-creatinine and s- cystatin C-based formulae. The prediction equations were evaluated according to recommendations in the NKF K/DOQI guidelines (2). ($n = 322$). Null hypothesis for the bias = 0.

	Mean (SD), ml/min/1.73 m^2	Bias	Precision	*P*	Accuracy (%) within 30%	Accuracy (%) within 50%	Relative difference (%), Mean (SD)	*R*
Estimated creatinine clearance								
Cockcroft–Gault/1.73 m^2								
Jelliffe	56.4 (30.1)	5.9	13.2	0.0001	68.6	84.8	26.9 (25.4)	0.90
GFR-creatinine MDRD	49.5 (26.9)	−0.9	13.0	0.2226	79.5	91.6	21.3 (18.9)	0.89
GFR-cystatin C	49.4 (27.0)	−1.0	12.9	0.1570	80.4	92.2	20.9 (19.3)	
Larsson*	58.4 (38.0)	7.9	24.4	0.0001	54.0	76.4	35.8 (50.0)	0.78
Hoek**	48.0 (24.7)	−2.4	10.3	0.0001	82.9	96.0	17.2 (14.6)	0.93
le Bricon**	54.8 (24.0)	4.4	10.4	0.0001	64.6	82.0	27.3 (27.1)	0.93
Grubb*	52.5 (36.2)	2.1	23.3	0.1022	71.7	90.1	24.4 (43.4)	0.77
Orebro-cyst DAKO*	52.3 (33.5)	1.8	18.5	0.0740	66.5	85.1	28.3 (34.8)	0.84
Orebro-cyst Gentian**	51.1 (30.7)	1.0	11.1	0.1100	82.0	95.0	18.0 (15.6)	0.93
GFR-creatinine/cystatin C combined								
Mean MDRD /Orebro-cyst Gentian	50.4 (27.6)	0	8.6	0.9774	87.0	97.2	14.9 (14.2)	0.95
Mean MDRD /Orebro-cyst DAKO	50.8 (28.3)	–0.4	11.5	0.5189	81.1	94.1	18.9 (20.1)	0.92

S-cystatin C according to turbidimetric determination using reagents from *DAKO and from **Gentian were used.

Table 4. Stages of chronic kidney disease according to GFR estimating equations based on s-creatinine and s-cystatin C

	Stage 1, GFR ≥ 90	Stage 2, GFR 60–89	Stage 3, GFR 30–59	Stage 4, GFR 15–29	Stage 5, GFR < 15	Total	Kappa statistics, κ (95% CI)
Measured GFR (number of patients in each stage)	39	75	114	81	13	322	
Estimated creatinine clearance							
Cockcroft—Gault	29 (74.4%)	47 (62.7%)	85 (74.6%)	37 (45.7%)	8 (61.5%)	206 (64.0%)	0.67 (0.60–0.75)
Jelliffe	21 (53.8%)	36 (48.0%)	85 (74.6%)	50 (61.7%)	10 (76.9%)	202 (62.7%)	0.67 (0.60–0.74)
GFR-creatinine							
MDRD	14 (35.9%)	43 (57.3%)	87 (76.3%)	47 (58.0%)	9 (69.2%)	200 (62.1%)	0.66 (0.59–0.73)
GFR-cystatin C							
Larsson	29 (74.4%)	37 (49.3%)	79 (69.3%)	49 (60.5%)	6 (46.2%)	200 (62.1%)	0.63 (0.56–0,70)
Hoek	20 (51.3%)	50 (66.7%)	89 (78.1%)	63 (77.8%)	2 (15.4%)	224 (69.6%)	0.71 (0.64–0.78)
le Bricon	25 (64.1%)	60 (80.0%)	93 (81.6%)	27 (33.3%)	0 (0%)	205 (63.7%)	0.65 (0.58–0.72)
Grubb	28 (71.8%)	43 (57.3%)	80 (70.2%)	57 (70.4%)	8 (61.5%)	216 (67.1%)	0.71 (0.64–0.78)
Orebro-cyst DAKO	28 (71.8%)	47 (62.7%)	76 (66.7%)	36 (44.4%)	11 (84.6%)	198 (61.5%)	0.67 (0.60–0.74)
Orebro-cyst Gentian	32 (82.1%)	53 (70.7%)	81 (71.1%)	55 (67.9%)	11 (84.6%)	232 (72.0%)	0.76 (0.68–0.83)
GFR-cystatin C/creatinine							
Mean MDRD/Orebro-cyst Gentian	25 (64.1%)	53 (70.7%)	92 (80.7%)	58 (76.9%)	10 (76.9%)	238 (73.9%)	0.78 (0.70–0.85)
Mean MDRD/Orebro-cyst DAKO	26 (66.7%)	57 (76.0%)	88 (77.2%)	53 (65.4%)	2 (15.4%)	226 (70.2%)	0.77 (0.69–0.84)

Number of patients (%) correct classified. Reference method is plasma clearance of iohexol. ($n = 322$). All GFR values in ml/min/1.73 $m^{2.}$ Kappa analysis was used to evaluate the agreement between stages.

Creating formulae for estimating GFR requires the use of a reference method and we have used the plasma clearance of iohexol for both the creatinine and cystatin C formulae [27]. Since the cystatin C formulae have been calculated using GFR related to a standardised body area, the eGFR results are presented as GFR in ml/min/1.73 m^2.

There are a great many studies that have validated estimated creatinine clearance (Cockcroft–Gault) and eGFR from plasma creatinine concentrations (MDRD) [28, 29]. Several methodological problems exist: the patient population studied, the analysis method and the calibration of the creatinine method, the different GFR reference methods used and the choice of the statistical method used for the comparison. Both Cockcroft–Gault and the original MDRD formulae are based on Jaffe's creatinine method. In many studies the comparison has involved only the use of a correlation coefficient which is not regarded as adequate. In its place bias, precision, accuracy and relative difference should be calculated in order to properly compare the formulae [2]. Because the studies have been constructed in different ways, it is difficult to compare data. MDRD is regarded as being less biased and is the creatinine-based formula which is recommended for follow-up of patients with CKD [30].

In previous reports with differing patient populations the MDRD formula was accurate within 30% for 84–91% of studied patients [31–34]. In our patient population (GFR 12–125 ml/min/1.73 m^2) the accuracy of the MDRD formula within 30% was lower, 80.4%. It should be pointed out that the creatinine method used in this study was the IDMS standardised Roche enzymatic method.

Several studies have suggested that s-cystatin C is a superior marker for renal function than creatinine [35,36], whereas others have questioned this [37]. S-cystatin C as a marker of GFR has been investigated in fewer studies.

The cystatin C formulae are dependent upon the particular analytical method used for determining this component. The importance of using a correct formula when calculating eGFR is obvious since different equations will give different estimates. A s-cystatin C concentration of 1 mg/l will give rise to calculated eGFRs from 76 to 99 ml/min/1.73 m^2 depending upon the formula used. The differences were greatest at the high and low cystatin C values, depending upon the power regression used. The fact that the relationship between 1/s-cystatin C and GFR is more linear for the Gentian method ($R = 0.9322$) compared with the DAKO method ($R = 0.8350$) strongly implies that the Gentian method is superior. Grubb *et al.* and Larsson [19,21,38] have taken this lack of linearity into account by using power equations to estimate GFR, which gives a good curve passing for the measuring interval of greatest interest. As can be seen, the different analysis methods give rise to different constants in the formula GFR = Cys-pr/S-Cys – CL-nr (Table 2). Calculation of CL-nr with our method using DAKO reagents gives rise to increasing values with increasing GFR, which is physiologically unlikely.

It is necessary to be able to adapt a published formula to account for differences in the analytical method used for determining cystatin C and even if the analytical method is the same, to compensate for differences between laboratories. Our formula can be used with cystatin C determination methods that have a linear relationship between 1/s-cystatin C and GFR. It is necessary to determine the intercept and slope of the regression line. The intercept can be estimated using a few samples from anuric dialysis patients and the slope using a few samples from patients with normal kidney function where GFR can be determined with a reference method.

The GFR estimates from both creatinine and cystatin C are associated with sources of errors. The variation in the production rate can be estimated and would appear to

be greater for creatinine than cystatin C. Creatinine has a variable tubular secretion and reabsorption, but a small non-renal clearance. Cystatin C, on the other hand has a greater non-renal clearance, which also appears to vary. The sources of error for estimating GFR from cystatin C and creatinine are distinctly different. Our study has shown that these two estimates have a similar accuracy. Thus it can be expected that combining GFR estimates from both these analytes should give rise to more accurate eGFR results than the eGFR results from the separate analytes. We have shown this in this study and this method has been in use in our laboratory for the last 2 years. Patients with a small muscle mass have grossly abnormal creatinine production which means that the GFR estimate from cystatin C is to be preferred over the GFR estimated from the mean. The classification results improve with the use of two measurements. However, for screening purposes the IDMS-based MDRD is probably sufficient.

Limitations in our study were that only adults were included and that the patient population was mainly North European.

In summary, estimating GFR using formulae based on s-creatinine or s-cystatin C alone was equally accurate according to the KDIGO and the NKF K/DOQI guidelines. A formula that combines both provided a greater accuracy. It is important to be aware of the differences between different methods for determining cystatin C when applying eGFR formulae. The choice of a cystatin C method where the relationship 1/S-cystatin C to GFR is linear is essential. An international calibrator for cystatin C would greatly improve its use as a marker for GFR. The use of cystatin C only as a determinator of eGFR does not yield improved accuracy over IDMS-based MDRD, and is more expensive.

Conflict of interest statement. None declared. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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