

Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population

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Measurement of blood concentrations of cystatin C (cysC), a cysteine protease inhibitor present in human plasma, has been suggested for use as an indicator of glomerular filtration rate (GFR) in a manner analogous to the use of plasma creatinine (SCR). In this study, cysC and SCR were measured in plasma from pediatric patients (4–19 years) with renal disease for whom a “gold standard” measurement of GFR via inulin clearance (C_{IN}) was available. The data analyses were divided into two age groups: group A (4–12 years, $n = 26$) and group B (12–19 years, $n = 34$). For both age groups, the linear correlation coefficient of $[cysC]^{-1}$ vs C_{IN} ($\text{mL}/\text{min}/1.73 \text{ m}^2$) ($r = 0.765$ for group A and $r = 0.869$ for group B) was less than that of the linear correlation coefficient of $[SCR]^{-1}$ vs C_{IN} ($r = 0.841$ for group A and $r = 0.892$ for group B). As a single measurement for detection of abnormal GFR, however, the optimum receiver-operator characteristic point for cysC measurement (for group A at $cysC > 1.2 \text{ mg/L}$, sensitivity = 80%, specificity = 91%; and for group B at $cysC > 1.4 \text{ mg/L}$, sensitivity = 87%, specificity = 100%) was numerically superior to that for SCR measurement (for group A at $SCR > 8.0 \text{ mg/L}$, sensitivity = 67%, specificity = 100%; and for group B at $SCR > 9.0 \text{ mg/L}$, sensitivity = 91%, specificity = 91%), using a reference value for normal GFR of $C_{IN} > 90 \text{ mL}/\text{min}/1.73 \text{ m}^2$. However, these differences were not statistically significant. CysC measurement appears to

be broadly equivalent to SCR measurement for estimation of GFR in pediatric patients.

Cystatin C (cysC)³ is a 122-amino acid, 13-kDa cysteine proteinase inhibitor that is an endogenous component of human plasma (1). CysC has been described as the product of a “housekeeping gene” that is expressed in all nucleated cells, because the production of this inhibitor appears to be stable and unmodulated (2). CysC is freely filtered by the glomerulus, and it is neither secreted nor is it reabsorbed as an intact molecule (1). Thus, measurement of cysC in plasma has been proposed as a means of estimating glomerular filtration rate (GFR) in a manner analogous to the use of plasma creatinine (SCR) (3–5).

The principle for use of a plasma analyte such as SCR for measurement of GFR is based on a steady-state mass balance, with the assumptions that the rate of appearance into the bloodstream is constant and balanced solely by the rate of filtration through the glomerulus, leading to elimination in the absence of tubular secretion or reabsorption. SCR is used widely as an analyte to estimate GFR on the basis of these assumptions, although the assumptions are not rigorously true: SCR can be secreted by renal tubules, and the rate of appearance into the bloodstream can be altered by changes in muscle mass. CysC appears to possess distinct advantages over SCR in each of these respects for estimation of GFR (1, 3–6). Thus it has been the object of several studies to develop procedures for cysC measurement and to examine its use as a marker of GFR in adults (3–5, 7–14).

The use of cysC measurement in pediatric populations, in which reliance on SCR measurements can be problematic because of low muscle mass, has not been examined. In this study, plasma cysC and SCR were measured in a

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³ Nonstandard abbreviations: cysC, cystatin C; GFR, glomerular filtration rate; SCR, plasma creatinine; and C_{IN} , inulin clearance.

pediatric population for comparison with GFR measured by inulin clearance (C_{IN}).

Materials and Methods

SAMPLES

This study was approved by the Washington University Human Studies Committee. Subjects were patients who were administered inulin to determine GFR for their clinical management at St. Louis Children's Hospital during the period of December 1995 to June 1996. Plasma specimens used for inulin measurements were saved under refrigeration for up to six weeks and then frozen for later measurement of cysC and SCR. Plasma cysC was presumed to be stable under these conditions, on the basis of previous reports (1, 7). Complete data sets for C_{IN} , plasma cysC, and SCR were available for 67 patients whose ages ranged from 1.8 to 18.8 years. Patients' renal diagnoses included obstructive nephropathy, reflux nephropathy, IgA nephropathy, renal dysplasia, chronic renal insufficiency, diabetes, hypertension, polycystic kidney disease, Alport's syndrome, and postrenal transplant.

ANALYTICAL METHODS

Measurement of inulin. Inulin in plasma or urine was measured using an automated enzymatic assay on the Cobas-FARA II analyzer (Roche Analytical Instruments) as previously described (15).

Measurement of cysC. CysC was measured with the Dako (Dako Corp.) particle-enhanced turbidimetric kit (7), using the Hitachi 717 (Boehringer Mannheim) analyzer.

Measurement of creatinine. Creatinine in plasma was measured using an automated picric acid assay on the Hitachi 717 analyzer according to the manufacturer's procedures.

Inulin clearance. Measurement of C_{IN} was performed according to the method and calculations of Cole et al. (16) and expressed per 1.73 m² body surface area.

Statistical analyses. Calculations and statistical analyses were performed according to standard formulae (17–19). For ROC plots, the "optimal point" was defined as the point having the greatest sum of sensitivity plus specificity.

Results

COMPARISON OF RELATIONSHIPS OF CYSC AND CREATININE CONCENTRATIONS TO C_{IN}

Plasma cysC concentrations ranged from 0.73 to 5.91 mg/L, and SCR concentrations ranged from 5.0 to 50 mg/L in this population. The high correlation ($r = 0.88$) between cysC and SCR (Fig. 1) supports the concept that cysC and SCR have similar properties as plasma markers of GFR (9).

The relationship between plasma cysC or creatinine

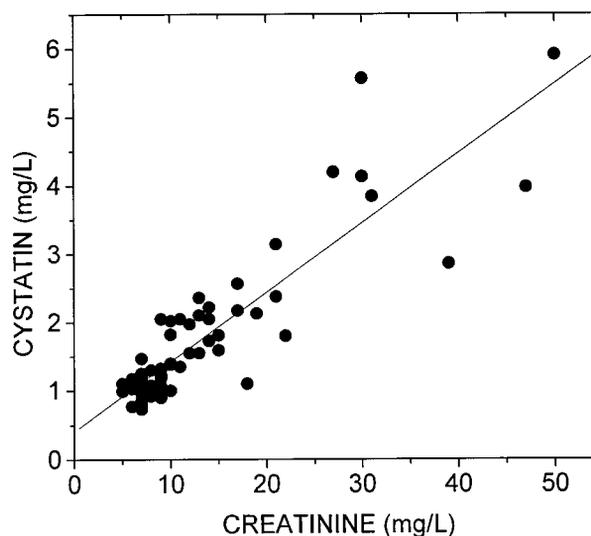


Fig. 1. Relation of plasma cysC to SCR.

Linear regression: $\text{cysC} = 0.102 \text{ SCR} + 0.412$; $r = 0.882$; $S_{y|x} = 0.522$; $n = 63$.

and GFR, as measured by C_{IN} , was analyzed according to the expectation for the ideal case that cysC and SCR should be inversely proportional to C_{IN} . The data for subpopulations divided according to age groups (group A, 4–12 years, $n = 26$; and group B, 12–19 years, $n = 34$) were analyzed using the SCR reference ranges for different age groups used at St. Louis Children's Hospital (Table 1). Three points in Fig. 1, representing patients <4 years, were not considered further because of the small sample size in this age group. The relationships between $[\text{cysC}]^{-1}$, $[\text{SCR}]^{-1}$, and C_{IN} for group A (4–12 years) and group B (12–19 years) are shown in Figs. 2 and 3, respectively. For both groups A and B, the linear correlation coefficient for $[\text{cysC}]^{-1}$ vs C_{IN} ($r = 0.77$ for group A and 0.87 for group B) was slightly less than that of the linear correlation coefficient for $[\text{SCR}]^{-1}$ vs C_{IN} ($r = 0.84$ for group A and 0.89 for group B). However, the difference in r was not significant for either age group ($P > 0.3$). As is apparent from Figs. 2 and 3, the regression-predicted GFRs based on cysC and SCR measurement are strongly correlated. Treating the regression-predicted GFRs from cysC and SCR measurements as paired measurements, the GFR estimates from the two analytes are indistinguishable on the basis of a t -test (for group A, $t = 0.004$, $P = 0.997$; for group B, $t = 0.036$, $P = 0.972$). The 95% confidence intervals for $C_{IN} = 90 \text{ mL/min/1.73 m}^2$ (the lower

Table 1. Pediatric reference ranges for creatinine at St. Louis Children's Hospital.

Patient age	Creatinine, mg/L
Newborn	3–10
0–4 years	2–4
4–12 years	3–7
12–18 years	5–10

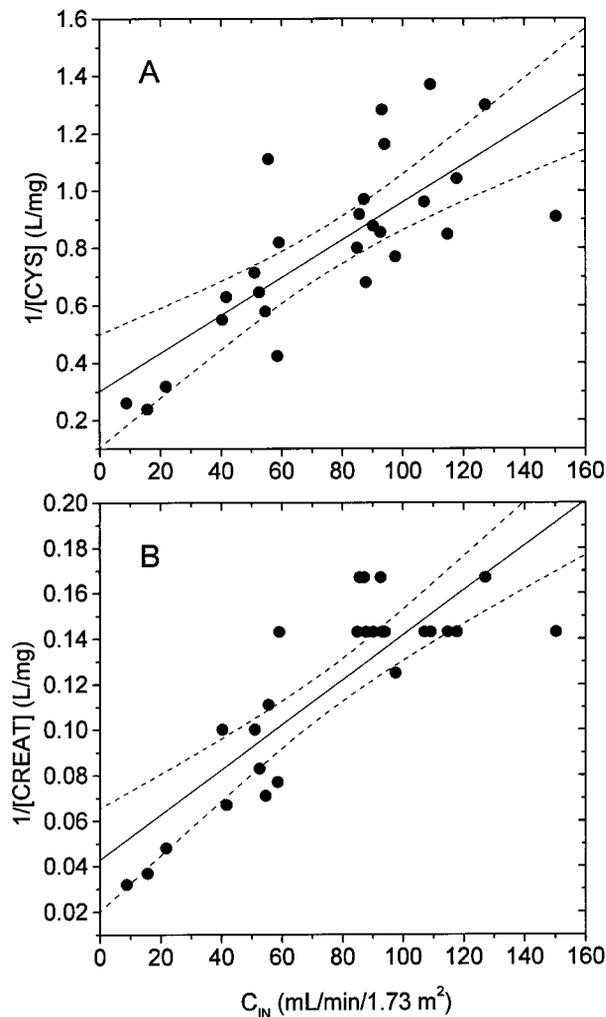


Fig. 2. Relation of (A) $[\text{cysC}]^{-1}$ and (B) $[\text{SCR}]^{-1}$ to C_{IN} for patient group A (4–12 years, $n = 26$).

Linear regressions: (A) $[\text{cysC}]^{-1} = 0.0066 C_{\text{IN}} + 0.30$; $r = 0.77$, $S_{y|x} = 0.20$; (B) $[\text{SCR}]^{-1} = 0.00099 C_{\text{IN}} + 0.043$; $r = 0.84$, $S_{y|x} = 0.023$. Dashed lines demarcate the 95% confidence interval for the dependent (y) variable.

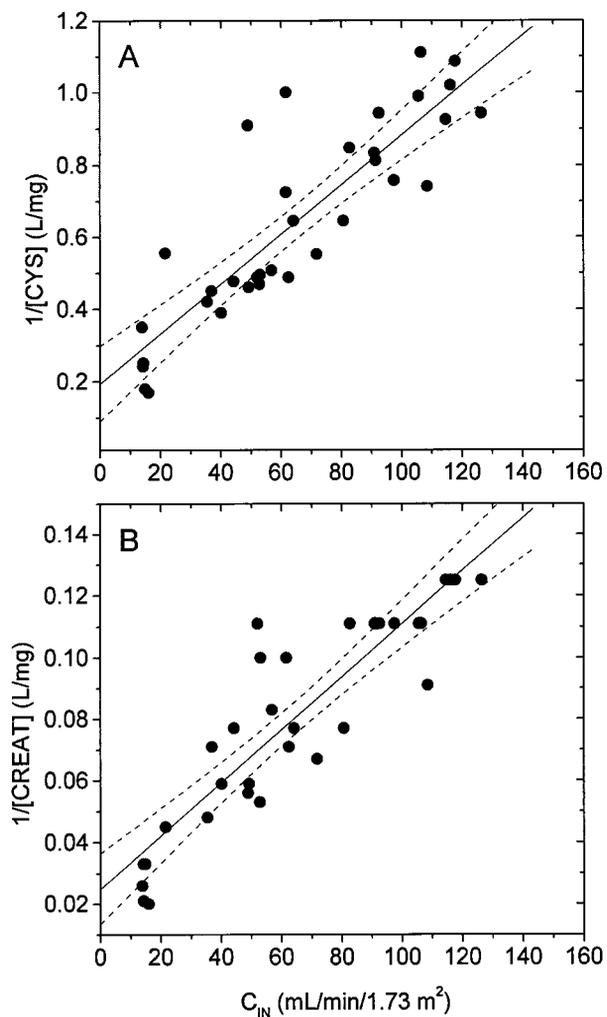


Fig. 3. Relation of (A) $[\text{cysC}]^{-1}$ and (B) $[\text{SCR}]^{-1}$ to C_{IN} for patient group B (12–19 years, $n = 34$).

Linear regressions: (A) $[\text{cysC}]^{-1} = 0.0069 C_{\text{IN}} + 0.19$; $r = 0.87$; $S_{y|x} = 0.14$; (B) $[\text{SCR}]^{-1} = 0.00086 C_{\text{IN}} + 0.025$; $r = 0.89$, $S_{y|x} = 0.015$. Dashed lines demarcate the 95% confidence interval for the dependent (y) variable.

reference value) obtained from regression analysis of C_{IN} vs $[\text{SCR}]^{-1}$ gave somewhat smaller ranges (for group A, $C_{\text{IN}} = 79.9$ – 100.1 mL/min/1.73 m²; for group B, $C_{\text{IN}} = 82.4$ – 97.6 mL/min/1.73 m²) than those obtained from the same analysis of C_{IN} vs $[\text{cysC}]^{-1}$ (for group A, $C_{\text{IN}} = 76.8$ – 103.2 mL/min/1.73 m²; for group B, $C_{\text{IN}} = 81.4$ – 98.6 mL/min/1.73 m²), but again these differences were not significant.

Four samples were excluded from the figures and from subsequent analyses because the correlation-predicted GFR (with or without these points included) from either the measured cysC or from the measured SCR was >160 mL/min/1.73 m². C_{IN} for each of these patients was actually <80 mL/min/1.73 m², suggesting either false-negative cysC or SCR values or erroneous measurement. Unfortunately, we were unable to repeat the measurements for these samples because of the small sample volumes.

COMPARISON OF SINGLE CYS C AND CREATININE MEASUREMENTS FOR PREDICTION OF GFR

The ROC curves of SCR and cysC measurements indicating an abnormal GFR were compared using a lower GFR reference value of 90 mL/min/1.73 m² (the lower reference value at St. Louis Children's Hospital); the results are summarized in Table 2. In group A ($n = 26$), there were 15 patients with GFR <90 mL/min/1.73 m² and 11 with normal GFR, whereas in group B ($n = 34$), there were 23 patients with GFR <90 mL/min/1.73 m² and 11 with normal GFR. For both age groups, the area-under-curve for cysC was not significantly different from that for SCR (Table 2). If the optimum cutoff for cysC or SCR is defined as the point at which the sum of sensitivity and specificity is greatest, test performance for cysC was numerically superior to that for SCR (Table 2); the differences, however, were not significant ($P > 0.56$ for all comparisons).

Table 2. Receiver-operator and test performance characteristics.

Age, years	Analyte	Cutoff ^a	Sensitivity	Specificity	AUC ^b	P	n
4–12 (Group A)	CysC	1.2 mg/L	0.80 (0.54–0.93) ^c	0.91 (0.62–0.98)	0.88 ± 0.07		26
	SCR	8.0 mg/L	0.67 (0.42–0.85)	1.00 (0.74–1.0)	0.79 ± 0.09	0.29	26
12–19 (Group B)	CysC	1.4 mg/L	0.87 (0.68–0.96)	1.00 (0.74–1.0)	0.94 ± 0.04		34
	SCR	9.0 mg/L	0.91 (0.73–0.98)	0.91 (0.62–0.98)	0.96 ± 0.03	0.99	34

^a Optimal upper reference value from ROC curve analysis (see text) using 90 mL/min/1.73m² as the lower reference value for GFR.

^b Area under ROC curve ± 1 SD.

^c Values in parentheses are 95% confidence intervals.

Discussion

In this study, plasma cysC and SCR were measured in parallel with C_{IN} as a measure of GFR in a population of pediatric patients with renal disease, and the characteristics of the relationship of cysC and SCR to C_{IN} were compared. As expected, there was a strong correlation between cysC and SCR, and there was a broadly similar relationship of the correlation between [cysC]⁻¹ and C_{IN} and the correlation based on [SCR]⁻¹ for two age groups (4–12 and 12–19 years). Although the SCR correlation to C_{IN} was slightly superior to that for the cysC correlation, the difference was not significant (*P* > 0.25 for both groups). The optimum ROC points for single-measurement determination of an abnormal GFR, using cysC and SCR measurements, were essentially equivalent, although cysC measurement showed somewhat greater sensitivity.

Correlation of cysC measurements with measurement of GFR has been reported in a number of previous studies by Grubb and coworkers (3, 4, 7, 8, 10). Grubb et al. (4) first reported the correlation (*r* = 0.75–0.77) of [cysC]⁻¹ concentrations with GFR measured by ⁵¹Cr-EDTA clearance, and also found a similar correlation (*r* = 0.73–0.75) for [SCR]⁻¹ vs ⁵¹Cr-EDTA clearance in renal patients ranging in age from 7 to 77 years. Stronger correlation to ⁵¹Cr-EDTA clearance (*r* = 0.87) together with a greater distinction from the plasma SCR correlation (*r* = 0.71) were reported in a more recent study using a particle-enhanced turbidimetric assay for cysC measurement in patients 8–81 years of age (7, 8). Similar numbers for the correlation of [cysC]⁻¹ vs ⁵¹Cr-EDTA clearance (*r* = 0.81) were reported in the most recent studies (9, 10), but the correlation using SCR was markedly lower (*r* = 0.50). The correlation of [cysC]⁻¹ vs C_{IN} in this study of pediatric patients (*r* = 0.77 for 4–12 years; and *r* = 0.87 for 12–19 years) is comparable with the correlations obtained in previous studies; however, we found better correlation of C_{IN} vs SCR (*r* = 0.84 for 4–12 years, and *r* = 0.89 for 12–19 years).

The diagnostic sensitivities and specificities for each analyte have also been reported previously (sensitivity = 71.4%, specificity = 95.1% for cysC; sensitivity = 52.4%, specificity = 91.8% for SCR) for a mixed pediatric and adult population, using a lower reference value for GFR of 72 mL/min/1.73 m² (10). Those results were similar to values reported using a GFR cutoff of 80 mL/min/1.73 m² (7). In this study, using pediatric patients and a GFR

cutoff of C_{IN} < 90 mL/min/1.73 m², we found greater sensitivity for cysC (sensitivity = 80%, specificity = 91% for 4–12 years; sensitivity = 87%, specificity = 100% for 12–19 years) but less distinction from SCR (sensitivity = 67%, specificity = 100% for 4–12 years; sensitivity = 91%, specificity = 91% for 12–19 years) than in these previous studies. The reasons for these differences may be related to the restriction of the age of the population used in this study. We found that the optimal cutoff values for cysC by ROC analysis ranged from 1.2 to 1.4 mg/L in these two pediatric age groups. Filler et al. (14) showed that cysC is not age-dependent in subjects < 20 years of age. These authors report a 97.5th percentile value for cysC of 1.38 mg/L and suggest that any value > 1.4 mg/L is suggestive of an abnormal GFR. The data presented here are in full accord with those findings.

Four samples were rejected when either the cysC or SCR value was so low so as to correspond to an unusually high and unrealistic GFR of > 160 mL/min/1.73 m². In these cases, the GFRs predicted using the other analyte were not abnormally high. Thus, the combined testing of cysC with SCR might be used as a check for the validity of the data, given the high overall degree of correlation between the two measurements. Notably, the deviations of the two measurements from the corresponding population regression lines are not correlated in sign (data not shown).

From the standpoint of clinical use, cysC measurement has the disadvantages that, unlike creatinine, its predicted GFR cannot be verified by a measurement of its clearance and that it is not readily available at present. Thus, cysC measurement is unlikely to replace creatinine measurement in routine clinical practice. However, given the equivalence of SCR and cysC for measurement of GFR, cysC might be useful for cases in which, for whatever reason, a verification of the creatinine measurement by measurement of the creatinine clearance is desired but cannot be obtained readily. This could be done using the same sample as that used for measurement of creatinine. Furthermore, as a single-sample measurement for estimation of GFR, cysC measurement apparently will not require corrections for age and weight as with use of creatinine in clinical practice (20, 21), although the potential for dependence on other determinants exists.

In summary, the results demonstrate that cysC is broadly equivalent to SCR measurement as a single-measure analyte for estimation of GFR in a pediatric population. CysC possesses the advantage that the concentration is not age-dependent (14), and thus a single reference value can be used as a cutoff for preliminary identification of an abnormal GFR. The lack of age dependence on CysC values may be a particular advantage in children under the age of 4 years, where small muscle mass results in low SCR values and increased imprecision in SCR measurements. Unfortunately, the number of patients in our study under the age of 4 was too small to assess this hypothesis. As with SCR measurement, however, clinical interpretation of cysC measurement is nonetheless better made in the context of serial measurements rather than from any single measurement. Newman et al. (10) demonstrated that cysC concentration shows a greater percentage change in response to near-normal decreases in GFR than does SCR. Given the essentially parallel utility of cysC measurements to SCR measurements in other respects, it may be these differences that will forward the routine use of cysC measurement in clinical management of renal patients.

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