Cystatin C Serum Concentrations Underestimate Glomerular Filtration Rate in Renal Transplant Recipients, Arend Bökenkamp,1 Michael Domanetzki,1 Raymund Zinck,1 Gerhard Schumann,5 Dennis Byrd,1,5 and Johannes Brodehl1

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Cystatin C, a cationic 13.3-kDa protein (1) has recently been described as a promising endogenous marker of glomerular filtration rate (GFR) (2) in both adults (2, 3) and children (4, 5). Correlation of serum cystatin C concentrations with the results of inulin and 51Cr-ethylendiaminetetraacetic acid clearance examinations was superior to serum creatinine (2–4).

Although patients with a wide range of renal function were examined, the potential effect of underlying disease on this correlation has not been studied yet. In a recent longitudinal study on 24 pediatric renal transplant recipients, cystatin C serum concentrations were found to underestimate GFR compared with the results of a 24-h creatinine clearance (6). To validate these findings, we extended our previous study on the correlation between inulin clearance (CIn) and serum cystatin C concentration in children (4).

One hundred and eighty-four children (87 girls, 97 boys) with a mean age of 11.2 ± 4.5 years (mean ± SD; range, 0.24–17.96 years) had undergone an inulin clearance examination between 1985 and 1995 at the Department of Pediatric Nephrology at Hannover Medical School. Forty-four of these (19 boys, 25 girls; mean age, 13.9 ± 3.2 years) had received a renal transplantation (RTx). Serum samples from CIn examinations had been stored at 20 °C for 6.9 ± 2.8 years (mean ± SD) until measurement of cystatin C, which has been shown to be stable for such extended periods of storage (4). Cystatin C was measured by particle-enhanced immunoturbidimetry (2, 3) using the Cystatin C PET-Kit (Dako) on a Hitachi 717 automated analyzer (Roche Diagnostics).

Because the GFR was subnormal in all of the RTx recipients, this group was compared with a control group of 56 non-RTx children (26 boys, 30 girls; mean age, 11.2 ± 4.8 years) also having a CIn below 84 mL·min⁻¹·1.73 m⁻². The disease spectrum included glomerulonephritis (n = 21), nephrotic syndrome (n = 2), tubulo-interstitial disease (n = 10), liver transplantation (n = 13), and miscellaneous disorders (n = 10). Serum samples from the control group had been stored under the same conditions as the RTx samples. CIn and cystatin C measurements in the control children were used to derive a formula for the estimation of GFR from serum cystatin C concentrations by linear regression analysis:

\[
GFR_{Cys}(mL \cdot min^{-1} \cdot 1.73m^{-2}) = 137 / \text{cystatin } C (mg/L) - 20.4 \quad (1)
\]

To study the effect of immunosuppression on the agreement between GFR

Cys and CIn independent of GFR, the deviation between GFR

Cys and CIn was normalized for GFR as:

\[
\text{relDev}_{Cys} (%) = \left( \frac{C_{In}}{GFR_{Cys}} \right) \cdot 100 / C_{In} \quad (2)
\]

where relDevCys is the relative deviation between GFR

Cys and CIn. A positive value of relDevCys indicates an underestimation of GFR from serum cystatin C measurements, i.e., an increase in serum cystatin C out of proportion to renal functional impairment.

Data are presented as means ± SE. Statistical analysis was performed with StatView Ver. 4.01 statistical software (Abacus Concepts) for Macintosh computers (Apple), using standard parametric tests as indicated. P values below 0.05 were considered statistically significant. Correlation was assessed by linear regression analysis and comparison of the slopes using parametric tests (7).

Cystatin C serum concentrations were significantly higher in the RTx group compared with the control group (2.5 ± 0.1 vs 2.1 ± 0.1 mg/L; P = 0.002, unpaired t-test), although CIn was not different between the two groups (50.5 ± 2.5 vs 53.4 ± 2.4 mL·min⁻¹·1.73 m⁻²; P = 0.423). Linear regression between the reciprocal of serum cystatin C and CIn (Fig. 1) showed a significantly lower slope of the regression line for the RTx patients (0.0046; 95% confidence interval, 0.0033–0.0059) than for the control group (0.0073; 95% confidence interval, 0.0060–0.0086; P = 0.002, two-sided t-test).

In the transplanted children, relDevCys differed significantly from zero (25.1% ± 2.7%; 95% confidence interval, 19.4–30.7%; P < 0.0001, two-tailed t-test). Linear regres-
sion analysis failed to show a significant correlation between relDevCys and cyclosporin A trough concentrations \((r = 0.12; P = 0.34)\), cyclosporin A dose \((\text{mg} \cdot \text{m}^{-2} \cdot \text{day}^{-1}; r = 0.13; P = 0.30)\) or prednisolone dose \((\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}; r = 0.188; P = 0.13)\) in those 66 children receiving immunosuppressive drugs.

The present study confirms our previous finding that cystatin C serum concentrations are increased in RTx compared with non-RTx children having the same GFR. Estimation of GFR from serum cystatin C concentrations by use of a regression formula derived in non-RTx patients with similar renal function led to an underestimation of \(~25\%\). This may account for some of the scatter observed in the Bland-Altman analysis in our previous report \((4)\), which demonstrated the limitations in estimating GFR from the reciprocal of cystatin C serum concentrations by linear regression with C\(_{\text{in}}\). If such regression formulae are to be used in clinical practice, care must be taken for a specific calibration in RTx patients.

Newman et al. \((8)\) have also noted increased cystatin C concentrations in spite of creatinine values within the reference interval in adult renal transplant recipients on discharge from hospital. In contrast, Plebani et al. \((9)\) found cystatin C concentrations within the reference interval in renal transplant recipients with C\(_{\text{in}}\) above 70 mL\(\cdot\)min\(^{-1}\)\(\cdot\)1.73 m\(^{-2}\). Even at a GFR of 10–15 mL\(\cdot\)min\(^{-1}\)\(\cdot\)1.73 m\(^{-2}\), they reported cystatin C concentrations of only 2.5 mg/L, which is one-half of the expected value for this degree of renal functional impairment \((2–4, 10)\).

In the present study, the slope of the regression line of the RTx group was significantly lower than the non-RTx group. Bearing in mind that the \(y\)-axis represents the reciprocal of cystatin C, this finding is suggestive of a constant increase in cystatin C concentrations independent of GFR.

Several mechanisms might explain the observed increase: (a) interference with the cystatin C assay either directly from the immunosuppressive drugs or from metabolic changes induced by immunosuppression; (b) tubulo-interstitial damage leading to a backleak of intact cystatin C into the circulation; or (c) impaired filtration of cystatin C because of increased protein binding.

We studied the effect of cyclosporin A and prednisolone as the major immunosuppressive drugs administered after RTx. None of the variables tested had a significant effect on cystatin C concentrations in our patients. In vitro experiments using dexamethasone in HeLa cells showed a substantial and dose-dependent increase in cystatin C production \((11)\). Most of our transplanted children were studied before discharge after successful RTx. At this time, the prednisolone dose had already been reduced to 4 mg\(\cdot\)m\(^{-2}\)\(\cdot\)day\(^{-1}\). The observed increase in serum cystatin C concentrations might reflect the higher steroid doses administered directly after transplantation \((12)\). If the early high-dose steroid therapy were to account for the observed increase in cystatin C, it would be expected to be transient, which has not been the case in our experience.

Addition of cyclosporin A to serum samples in vitro failed to demonstrate any interference with the assay (data not shown). Hypercholesterolemia and hypertriglycerideremia are common findings after RTx \((13)\). Lipemia (triglyceride concentration >10 mmol/L) has been shown to reduce cystatin C recovery in vitro \((2)\). This eliminates RTx-associated hyperlipidemia as an explanation for the observed increase in cystatin C.

Like other low-molecular weight proteins, filtered cystatin C is reabsorbed and catabolized in proximal renal tubular cells \((14, 15)\). With severe tubular injury, a backleak of intact cystatin C into the circulation is conceivable. This should also apply to the patient group with documented tubulo-interstitial disease. This group, however, did not have increased serum cystatin C (data not shown).

Newman et al. \((2)\) described three outliers with increased cystatin C concentrations, one of which was from a renal transplant recipient (the other two patients had autoimmune disease). Rheumatoid factor does not interfere with the immunoturbidimetric assay for cystatin C \((2, 3)\) in vitro. Immunoglobulins may, however, form high-molecular weight complexes with cystatin C, thus impairing glomerular filtration (Anders Grubb, Lund, Sweden, personal communication). Because the grafted kidney induces an antibody response \((16)\), the observed interaction might be attributable to impaired filtration of complexed cystatin C in allograft recipients. This hypothesis should apply to all kinds of organ transplantation but still remains to be tested.

In conclusion, cystatin C serum concentrations are increased in RTx children compared with non-RTx children having the same GFR. Further study is needed to elucidate the precise mechanism underlying this observation and to test whether it applies to other kinds of organ transplantation as well.

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References
Comparison of Tacrolimus Concentrations Measured by the IMx Tacrolimus II vs the PRO-TRAC II FK506 ELISA Assays, Zhimin (Tim) Cao,1 Mark W. Linder,1* Anthony W. Jeffons,2 Glenda Brown,1 and Roland Valdes, Jr.1
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Contemporary practice in the therapeutic monitoring of the immunosuppressant tacrolimus has required increasingly rapid and more sensitive assays. To this end, the two most commonly used immunoassay formats, IMx Tacrolimus II (Tacrolimus II) and PRO-TRAC II™ FK506 (ProTrac II) have undergone extensive revision, leading to changes in the measured drug concentration relative to the original assay format (1–3). Multiple studies have compared the original microparticle enzyme immunoassay (MEIA) and second-generation Tacrolimus II methods [e.g., Refs. (2, 3)], one report has compared the original MEIA and the second-generation ProTrac II method (1), and two preliminary reports have compared the ProTrac II method with the Tacrolimus II method (4, 5). In each of these reports, the ProTrac II method consistently yielded lower tacrolimus concentrations than the Tacrolimus II assay. The aim of this study was to further characterize the correlation between the Tacrolimus II method and the ProTrac II ELISA.

Tacrolimus II controls were kindly provided by Abbott Laboratories (Abbott Park, IL). Pure tacrolimus powder was a courtesy of Fujisawa USA (Chicago, IL).

Tacrolimus stock solutions in whole blood at concentrations of 5.0, 10.0, and 20.0 μg/L were prepared from tacrolimus powder. Tacrolimus powder was initially dissolved in methanol, followed by sequential dilution with drug-free human whole blood. Tacrolimus assays were performed according to manufacturers instructions.

Correlation data were analyzed by Deming regression [Medsnap program (6)]. Differences between the two methods were analyzed by the methods of Bland and Altman (7, 8).

Fig. 1. Correlation between the ProTrac II and Tacrolimus II methods (A) and differences in log tacrolimus vs log average tacrolimus concentration (B).

(A), whole blood specimens (n = 55) from 39 patients who had heart (n = 9), lung (n = 9), kidney (n = 7), liver (n = 10), or bone marrow (n = 4) transplants were assayed for tacrolimus using the ProTrac II and Tacrolimus II assays. Analysis by Deming regression returned the mathematical best fit of the data: Tacrolimus II = 1.18 (ProTrac II) + 2.2 μg/L. (B), difference in log measured tacrolimus concentration (ProTrac II − Tacrolimus II) plotted against the log average tacrolimus concentration to correct for proportional bias when determining the mean difference between methods. The antilog of the mean ± SD difference in log concentration (−0.172 ± 0.089) is 0.84 ± 0.15, indicating a mean difference of 16% (range, 0–29%).