BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH
www.bjournal.com.br

ISSN 0100-879X
Volume 42 (12) 1119-1247 December 2009

BIOMEDICAL SCIENCES
AND
CLINICAL INVESTIGATION

Braz J Med Biol Res, December 2009, Volume 42(12) 1225-1229

Cystatin C and renal function in pediatric renal transplant recipients

M.C.P. Franco, S.S. Nagasako, P.G. Machado, P.C.K. Nogueira, J.O.M. Pestana and R. Sesso

Brazilian Journal of Medical and Biological Research (2009) 42: 1225-1229
ISSN 0100-879X

Cystatin C and renal function in pediatric renal transplant recipients

M.C.P. Franco[1], S.S. Nagasako[2], P.G. Machado[1], P.C.K. Nogueira[2], J.O.M. Pestana[1] and R. Sesso[1]

[1]Disciplina de Nefrologia, Departamento de Medicina, [2]Departamento de Pediatria, Universidade Federal de São Paulo, São Paulo, SP, Brasil

Abstract

In clinical practice, the glomerular filtration rate (GFR) is often determined with serum creatinine. However, studies have shown cystatin C to be a better parameter for the diagnosis of impaired renal function. We compared GFR estimated by plasma cystatin C with GFR estimated by serum creatinine in a sample of 50 pediatric renal transplant recipients and 24 healthy children. The correlation between GFR estimated by serum creatinine and by cystatin C was significant (r = 0.75; P < 0.001, Pearson's correlation); however, in pediatric kidney transplant recipients, the GFR was 6.7 mL/min lower when determined using cystatin C rather than serum creatinine. Moreover, using GFR estimated by cystatin C we found that 42% of the pediatric kidney transplant recipients had an estimated GFR <60 mL·min^{-1}·1.73 (m^2)$^{-1}$, whereas when GFR was estimated by the serum creatinine formula only 16% of the children had values below this cutoff point indicative of chronic kidney disease (P < 0.001). We conclude that, in pediatric kidney transplant recipients, estimation of GFR yields lower values when cystatin C is used rather than serum creatinine.

Key words: Kidney transplant; Cystatin C; Creatinine; Glomerular filtration rate

Introduction

Monitoring of kidney graft function is mandatory for the detection of acute or chronic rejection and for the accompanying immunosuppressive therapies (1,2). Glomerular filtration rate (GFR) is considered to be the best marker of renal function, and serum creatinine is the biochemical parameter most commonly used to estimate GFR in routine practice. However, there are some shortcomings regarding the use of this parameter. Factors such as lean muscle mass and protein intake can influence serum creatinine, leading to an inaccurate estimation of GFR (2-4).

Cystatin C (CysC) is a nonglycosylated protein belonging to the cysteine protease inhibitors and is produced at a constant rate in all nucleated cells, removed from blood plasma by glomerular filtration and reabsorbed by the renal tubules (5,6). Moreover, its concentration is not influenced by diet, age, gender, lean muscle mass, and/or infections (7,8). Studies have suggested that CysC is a better marker of GFR than serum creatinine (Scr) (9-11). Nonetheless, there are contradictory reports about the value of CysC as a marker for GFR, particularly in pediatric renal transplantation (12,13). Several studies have been conducted to develop procedures for the estimate of GFR from CysC and the use of CysC levels as a GFR measure is widespread in clinical practice. Some predictive equations have been derived from the data of pediatric patients to estimate GFR from serum CysC concentration (14). However, only one has been validated in a separate cohort of pediatric renal transplant recipients (15). Accordingly, the objective of the present study was to evaluate and compare the performance of plasma CysC and a CysC-based eGFR equation to Scr and an Scr-based eGFR equation in a sample of pediatric renal transplant recipients, and compare these data with those obtained for a sample of children from the general population.

Material and Methods

The study was carried out on 50 renal transplant (RTx) children (24 boys and 26 girls; mean age, 12.6 ± 0.4 years), and who were followed at the Kidney and Hypertension Hospital, São Paulo, Brazil. These children were identified consecutively in the local ambulatory files among those who

Correspondence: M.C.P. Franco, Disciplina de Nefrologia, Universidade Federal de São Paulo, Rua Botucatu, 740, 04023-900 São Paulo, SP, Brasil. Fax: +55-11-5573-9652. E-mail: mdcfranco@nefro.epm.br

Received May 29, 2009. Accepted October 15, 2009. Available online October 30, 2009. Published December 4, 2009.

were between 12-18 months post-transplantation and had stable renal function. Patients were excluded for the following reasons: the legally responsible person was unable or unwilling to provide informed consent, hospitalization at the time of the study, presence of systemic infection requiring antibiotic therapy, clinically diagnosed chronic rejection, and a recent decline of renal function. Other clinical characteristics of the RTx children and the immunosuppressive drugs used at the time of follow-up evaluation are reported in Table 1. The control group consisted of 24 healthy children (11 boys and 13 girls; mean age, 10.5 ± 0.4 years) living in the community in the same region from which the cases came. None of the control children had a history of urinary tract infection or of renal diseases. In addition, none of the children in the study had clinical signs of thyroid dysfunction. The study was approved by the Ethics Committee of the Federal University of São Paulo and written informed consent was obtained from one of the parents of each child enrolled in the study.

Renal function assays

All children provided a blood sample, which was collected in the morning following an overnight fast. For CysC assays, aliquots of heparinized plasma were centrifuged at 1500 *g* for 5 min at 4°C and stored at -80°C. For Scr the aliquots were centrifuged at 1500 *g* for 20 min at 4°C and immediately processed in the Clinical Laboratory of São Paulo Hospital. CysC was measured with an immunoparticle kit (Dako Corp., Denmark) using an immunoturbidimetric assay. The range of detection of the assay is 0.3 to 7.5 mg per liter, with a reported reference range for young, healthy persons of 0.55 to 1.15 mg/L. We estimated GFR based on CysC (eGFRcys) using the equation described by Zappitelli et al. (15) as follows: $75.94 / \text{CysC [mg/L]}^{-1.17} \times 1.2$ (if renal transplant). Scr was measured using an automated picric acid assay with the Hitachi 717 analyzer according to manufacturer instructions in the Clinical Laboratory of São Paulo Hospital. The GFR based on Scr (eGFRcr) was estimated with the use of the Schwartz formula (16): k x height (cm) / Scr [mg/dL]; k = 0.55 in children up to 13 years of age. In addition, eGFR was adjusted for a body surface area divided by 1.73 m^2.

Table 1. Clinical characteristics of the 50 pediatric renal transplant recipients.

Characteristic	
Living donor	17 (34%)
Mean age at transplantation (years)	11.2 ± 0.5
Donor age (years)	22.8 ± 2.3
Acute rejection	9 (18%)
Delayed graft function	8 (16%)
Cold ischemia time (min)	887 ± 92.4
Renal artery stenosis	10 (20%)
Prior time on chronic dialysis (months)	18.5 ± 1.9
Causes of renal disease	
Glomerulonephritis	15 (30%)
Uropathy	16 (32%)
Undetermined cause/others	16 (32%)/3(6%)
Immunosuppressive therapy*	
Azathioprine	22 (44%)
Mycophenolate mofetil	27 (54%)
Cyclosporine A	9 (18%)
Tacrolimus	43 (86%)

Data are reported as number with percent in parentheses or means ± SEM. *All children were taking prednisone at a mean dose of 0.10-0.15 mg·kg body weight^{-1}·day^{-1}.

Statistical analysis

All continuous variables were examined for normality with the Kolmogorov-Smirnov test. The chi-square test (or the Fisher exact test where appropriate) and the McNemar symmetry chi-square test were applied for comparison of proportions. The Student *t*-test was used to compare mean values of continuous variables between two groups. Correlation between continuous variables was determined by the Pearson correlation coefficient. Analysis of covariance was used to compare the mean values of CysC and eGFRcys between RTx and control groups, with adjustment for potential confounding variables. Analyses were also performed by stratifying GFR into <60 and ≥60 mL·min^{-1}·1.73 $(m^2)^{-1}$ since this level has been recommended as the cutoff to define chronic renal failure (17). Data are reported as means ± SEM. Statistical tests were two-tailed and the level of significance was set at $P < 0.05$. All data were analyzed using the statistical program SPSS 11.0 for Windows.

Results

The demographic, anthropometric and clinical characteristics of the participants are shown in Tables 1 and 2. The mean age of the transplant recipients at the time of transplantation was 11.2 years (range: 3-16 years) in the RTx population and 22.8 years (range: 3-56 years) among the donors. Twenty-seven donors were adults and 23 donors were under 15 years old. The etiology of renal failure was uropathy (32%), glomerulonephritis (30%), and undetermined or other causes (38%). Live donor grafts were used in 34% of patients, adult cadaver grafts in 20% and pediatric cadaver grafts in 46%. Arterial hypertension was detected in 33 children before renal transplant, with 30 continuing to be hypertensive after the transplant.

Concentrations of both CysC and Scr were significantly higher in the RTx children compared with the controls (Table 2). In addition, eGFRcys and eGFRcr were significantly lower in RTx children than in controls (Table 2). In an analysis of covariance with adjustment for age and body weight, the results remained basically the same: mean

[95% confidence interval (CI)] for CysC, 1.54 mg/L (1.44 to 1.63) *vs* 0.66 mg/L (0.53 to 0.80, P < 0.001) for RTx and controls, respectively, and for eGFRcys, 66.3 mL/min (61.0 to 71.5) *vs* 125.9 mL/min (118.2 to 133.7, P < 0.001) for RTx and controls, respectively. CysC levels were significantly correlated with Scr levels (r = 0.74, P < 0.001) and eGFRcr (r = -0.80, P < 0.001). None of the anthropometric variables correlated significantly with plasma CysC levels (age: r = 0.18, P = 0.21, weight: r = 0.20, P = 0.18; height: r = 0.16, P = 0.17).

Divergent results were observed for Scr levels, since this marker correlated positively with age (r = 0.41, P < 0.001), height (r = 0.36, P = 0.002) and weight (r = 0.38, P = 0.001). Further analysis of the overall group was performed to test whether CysC and Scr varied by gender, and no significant difference between girls and boys was noted for either marker (CysC: 1.3 ± 0.09 and 1.2 ± 0.08 mg/L, P = 0.31, respectively; Scr: 1.0 ± 0.06 and 1.0 ± 0.058 mg/L, P = 0.87).

Because some recent studies have suggested that cyclosporine A (CsA) could promote a decrease in CysC levels, we determined the concentration of CysC and creatinine of the children who received this immunosuppressive drug. No significant differences were detected in the levels of CysC (with CsA: 1.7 ± 0.20 and without CsA: 1.5 ± 0.05) or creatinine (with CsA: 1.3 ± 0.19 and without CsA: 1.1 ± 0.04) between children receiving or not this treatment.

Although GFR estimated by Scr and CysC showed a significant correlation (r = 0.75, P < 0.001), in RTx recipients mean eGFRcys was significantly lower than eGFRcr (66.1 ± 2.0 *vs* 72.8 ± 2.2 mL/min), corresponding to a 10.1% increase in eGFR when the latter was employed. Using the eGFRcys equation, we found that 42% (95%CI: 28 to 56%; N = 21) of the children had a significant degree of impairment of renal function (GFR <60 mL·min^{-1}·1.73 (m^2)$^{-1}$). On the other hand, when we used the eGFRcr we observed that only 16% (95%CI: 6 to 26%; N = 8) of the children had GFR values <60 mL·min·1.73 (m^2)$^{-1}$ (P < 0.001). Therefore, 62% (13/21) of the children with a considerably reduced eGFRcys had a 'normal' eGFRcr estimated by the Schwartz formula.

In further analyses, we tested all demographic and clinical variables available in relation to eGFRcys (examined as a continuous or categorical (<60 or ≥60 mL/min) variable); however, we were unable to detect statistically significant associations between any of the independent variables and eGFRcys.

Table 2. Renal function tests in the study groups.

Characteristic	RTx children (N = 50)	Control children (N = 24)
Age (years)	12.6 ± 0.4	10.5 ± 0.4*
Weight (kg)	41.3 ± 2.1	32.6 ± 2.4*
Height (cm)	143.7 ± 2.5	137.3 ± 2.7
Creatinine (mg/dL)	1.13 ± 0.05	0.72 ± 0.01*
Range	0.62-2.48	0.60-0.80
eGFRcr (mL·min^{-1}·1.73 (m^2)$^{-1}$)	72.8 ± 2.20	105.3 ± 2.2*
Range	29.9-101.1	91.1-129.3
Cystatin C (mg/L)	1.55 ± 0.05	0.64 ± 0.03*
Range	1.02-2.51	0.47-1.05
eGFRcys (mL·min^{-1}·1.73 (m^2)$^{-1}$)	66.1 ± 2.03	126.3 ± 4.9*
Range	40.2-93.2	75.6-164.8

Data are reported as means ± SEM. RTx = renal transplant; eGFRcr = glomerular filtration rate estimated by creatinine; eGFRcys = GFR estimated by cystatin C. To convert serum creatinine in mg/dL to µmol/L, multiply by 88.4. *P < 0.05 compared to RTx children (Student *t*-test).

Discussion

Knowledge of GFR is of crucial importance in the management of pediatric renal transplant patients, in whom an accurate measurement of GFR is challenging. Determination of GFR with high accuracy requires the use of invasive techniques based on measuring the plasma clearance rate of injected substances that are exclusively excreted via glomerular filtration (18). However, these techniques are time-consuming, expensive, and not entirely free of risk for the patient. Thus, the measurement of endogenous blood substances to estimate GFR is a common practice, and Scr is the metabolite most commonly used for this purpose, although several drawbacks have been identified (2). Several studies have suggested that plasma CysC might be used as a GFR marker (9-11).

The data of the present study show important differences when using the Zappitelli and the Schwartz equations (15,16) to assess renal function in renal transplanted children. In fact, we observed that 62% of the children classified as having an important reduction of renal function using eGFRcys were classified as normal when GFR was estimated by the Schwartz formula. Using the cut-off point of <60 mL·min·1.73 (m^2)$^{-1}$, a remarkably lower percentage of RTx children were classified as having values below these levels by the Schwartz than by the Zappitelli formula. Based on previous studies reporting a greater precision of GFR estimation using CysC instead of Scr, our data suggest that the former equation may be able to correctly detect more patients with impaired renal function. These findings have important implications: 1) the classification of reduced renal function (and chronic kidney disease) in RTx children varies according to the method used to estimate GFR, 2) it seems that more patients will be classified as having chronic renal disease using a CysC-based than an Scr-based equation, 3) if these findings are confirmed, more patients could benefit from an early intervention to prevent a further decrease in renal function using the eGFRcys equation. The findings of this study, taken together with other reports, appear to support the more promising use of CysC as a possible marker

of preclinical or early kidney disease among persons with Scr-based eGFR in the 'normal' range (≥60 mL·min·1.73 $(m^2)^{-1}$) but with elevated levels of CysC. Some limitations of this study are the relatively small sample size, the lack of a direct measure of GFR, and we have not investigated predictors of lower eGFRcys.

The present report showed a good correlation between CysC and both Scr levels and eGFRcr, but we did not find any correlation of CysC with age, gender or other anthropometric parameters (7,8). In fact, CysC production in the body is a stable process that is not influenced by renal conditions, increased protein catabolism, or dietetic factors. Moreover, it does not change with age or muscle mass like Scr does (19). The effect of age on CysC has been reported. It has been demonstrated that circulating levels of CysC reach adult values by the 1st year of life and remain constant up to the age of 50 years, when it rises significantly due to the physiological aging of renal function (14). CysC may have limitations as a marker of renal function, since there are potential factors other than GFR that have been reported to affect serum levels of CysC including older age, male gender, smoking, higher weight, higher levels of C-reactive protein, and thyroid dysfunction (8,20). Moreover, recent studies have reported that CysC levels are affected by treatment with high steroid doses (21-24). Higher serum CysC levels and underestimation of GFR have been found in children on glucocorticoid therapy in the immediate post-transplant period and in situations of acute rejection. On the other hand, the effect of other immunosuppressive medications such as tacrolimus, mycophenolate mofetil or azathioprine on CysC concentration has not been reported, except for cyclosporine A, which appeared to promote a decrease in CysC levels (24). In our study, patients were evaluated more than one year after the transplant when the steroid dose used was much lower (0.1 $mg \cdot kg^{-1} \cdot day^{-1}$), and was unlikely to have influenced CysC measurements. In addition, we did not detect any significant effect of other immunosuppressive drugs on CysC levels.

The recent literature strongly suggests that CysC will have a role in assessing renal function in certain groups of patients for whom the disadvantages of Scr have become apparent. Pediatric renal transplant recipients are a group that would greatly benefit from studies better defining eGFR equations. In our study, the most frequently used Scr-based equation appeared to detect less RTx children with a significantly reduced renal function than the CysC-based equation. If CysC proves to be an earlier marker of renal dysfunction in RTx children it will be an important tool to improve the management of these children.

Acknowledgments

Research supported by FAPESP (#04/10342-7). R. Sesso receives a research grant from CNPq.

References

1. McCullough PA. Cardiorenal risk: an important clinical intersection. *Rev Cardiovasc Med* 2002; 3: 71-76.
2. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; 38: 1933-1953.
3. James GD, Sealey JE, Alderman M, Ljungman S, Mueller FB, Pecker MS, et al. A longitudinal study of urinary creatinine and creatinine clearance in normal subjects. Race, sex, and age differences. *Am J Hypertens* 1988; 1: 124-131.
4. Swan SK. The search continues - an ideal marker of GFR. *Clin Chem* 1997; 43: 913-914.
5. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 1992; 38 (Suppl 1): S20-S27.
6. Grubb AO. Cystatin C - properties and use as diagnostic marker. *Adv Clin Chem* 2000; 35: 63-99.
7. Hari P, Bagga A, Mahajan P, Lakshmy R. Effect of malnutrition on serum creatinine and cystatin C levels. *Pediatr Nephrol* 2007; 22: 1757-1761.
8. Knight EL, Verhave JC, Spiegelman D, Hillege HL, De Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; 65: 1416-1421.
9. Newman DJ, Thakkar H, Edwards RG, Wilkie M, White T, Grubb AO, et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. *Kidney Int* 1995; 47: 312-318.
10. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, et al. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. *Clin Chem* 1994; 40: 1921-1926.
11. Oddoze C, Morange S, Portugal H, Berland Y, Dussol B. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. *Am J Kidney Dis* 2001; 38: 310-316.
12. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C serum concentrations underestimate glomerular filtration rate in renal transplant recipients. *Clin Chem* 1999; 45: 1866-1868.
13. Krieser D, Rosenberg AR, Kainer G, Naidoo D. The relationship between serum creatinine, serum cystatin C and glomerular filtration rate in pediatric renal transplant recipients: a pilot study. *Pediatr Transplant* 2002; 6: 392-395.
14. Herget-Rosenthal S, Bokenkamp A, Hofmann W. How to estimate GFR-serum creatinine, serum cystatin C or equations? *Clin Biochem* 2007; 40: 153-161.
15. Zappitelli M, Parvex P, Joseph L, Paradis G, Grey V, Lau S, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. *Am J Kidney Dis* 2006; 48: 221-230.
16. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. *J Pediatr* 1984; 104: 849-854.

17. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S14-S266.
18. Laterza OF, Price CP, Scott MG. Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 2002; 48: 699-707.
19. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C - a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics* 1998; 101: 875-881.
20. Wasen E, Suominen P, Isoaho R, Mattila K, Virtanen A, Kivela SL, et al. Serum cystatin C as a marker of kidney dysfunction in an elderly population. *Clin Chem* 2002; 48: 1138-1140.
21. Risch L, Herklotz R, Blumberg A, Huber AR. Effects of glucocorticoid immunosuppression on serum cystatin C concentrations in renal transplant patients. *Clin Chem* 2001; 47: 2055-2059.
22. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B. Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. *Clin Chem* 2002; 48: 1123-1126.
23. Mendiluce A, Bustamante J, Martin D, Santos M, Bustamante R, Pascual P, et al. Cystatin C as a marker of renal function in kidney transplant patients. *Transplant Proc* 2005; 37: 3844-3847.
24. Xu H, Lu Y, Teng D, Wang J, Wang L, Li Y. Assessment of glomerular filtration rate in renal transplant patients using serum cystatin C. *Transplant Proc* 2006; 38: 2006-2008.