Macedonian pharmaceutical bulletin, **51**, (1,2) 35-40 (2005)
ISSN 1409 - 8695
UDC: 616.153.96:616.61-089.843

Original scientific paper

Serum Cistatin C in patients with delayed graft function

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Received November 2005, accepted April 2006

Abstract

Despite recent studies showing that serum Cystatin C(CysC) is a better marker for glomerular filtration rate (GFR) than the ubiquitously used creatinine, the clinical utility of this remains to be evaluated. This marker is very sensitive for alograft function after renal transplantation. Concentration of CysC was compared with that of creatinine. Decreased renal function was followed in 64 transplanted patients. Plasma CysC significantly correlated ($r=0.625$, $p<0.001$) with creatinine in healthy controls. In these patients the mean plasma creatinine and Cystatin C concentrations were: 81+/-13 mmol/L,0.90 +/-0.22 mg/L, respectively. Plasma Cystatin C and creatinine significantly correlated throughout the post-transplantation period ($r=0.686$, $p<0.001$), but we confirmed differences between kinetics of these parameters. In the first four days after transplantation the CysC concentration was normalized faster than creatinine concentration. Development of acute rejection episode (between 5 and 7 days) showed high sensitivity and specificity of the changes of CysC compared with those of creatinine.

Key words: renal transplantation, delayed graft function, cystatin C

Introduction

Cystatin C (Cys C) is a nonglycosylated basic protein (13.36 kDa) and can be found in a variety of biologic fluids (1). CysC serum concentration is not influenced by gender, inflammation, or lean tissue mass and is regarded to be mainly determined by glomerular filtration rate (GFR) (2, 3). Cystatin C has been described as meeting many of the characteristics of an ideal GFR marker (e.g., endogenously produced at a constant rate, freely filtered in the glomerulus, neither reabsorbed nor secreted in the renal tubule, not extrarenally eliminated) and has been reported to be at least as accurate as the commonly used serum creatinine to detect impaired renal function in various patient groups, including renal transplant patients (4-11).

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In recent literature, cystatin C has been advocated as a new and more accurate estimate of GFR (1).Cystatin C is a 13-kDa endogenous cysteine proteinase inhibitor produced by all nucleated cells at a constant rate and broken down completely in the renal tubuli (12). Cystatin C concentrations are independent of age and body weight, and there is no need for urine collection for clearance estimations. Furthermore, serum concentrations of cystatin C are not influenced by malignancy or inflammation. In contrast, the often-used serum creatinine concentration is supposedly influenced by dietary intake, renal tubular metabolism, age, and variations in muscle mass. There are also various analytical difficulties with the widely used Jaffe colorimetric assay for creatinine. A slight decrease in GFR has been found in patients with hypothyroidism, which improved significantly after treatment (2,3). We wondered whether cystatin C would also be a good marker of renal function in case of thyroid dysfunction. Thyroid hormones have metabolic effects and thus, thyroid state could influence plasma cystatin C concentrations.

Patients are at risk of acute damage of the transplanted kidney because of rejection or toxicity from immunosuppressant therapy. Earlier detection of renal damage may lead to more effective intervention. In a preliminary study, LeBricon et al. (13) first suggested that CysC was more sensitive than serum creatinine(SCr) for detecting decreases in GFR and delayed graft function in renal transplant patients. As in most studies, plasma CysC measurements correlated well with SCr and creatinine clearence (CrCl). However, in the three cases of acute renal rejection that were confirmed by biopsy, the increase in plasma CysC values was more pronounced than that observed for SCr.

After renal transplantation, plasma (or serum) creatinine is the most common marker for assessment of allograft function. In a steady-state muscular mass balance, the plasma creatinine concentration is assumed to reflect glomerular filtration rate (GFR) (14). However, plasma creatinine is far from being an ideal marker of GFR, despite its convenience and low cost (15) Plasma creatinine suffers a high degree of interindividual variability related to sex, age, body composition, and dietary factors (16). With altered renal function, the plasma creatinine concentration increases only when the GFR is reduced by >50%. Furthermore, secretion or reabsorption of creatinine by the renal tubule is highly unpredictable, thus leading to under- or overestimation of GFR (15).

Numerous drugs and endogenous substances also interfere with the measurement of creatinine by the Jaffé technique or enzymatic methods, leading to falsely high or low creatinine values (17).

Experimental

Samples

Sixty-four patients with end-stage renal disease undergoing renal transplantation were included in this study. Primary diagnosis of the investigated patients was: chronic interstitial nephropathy (n = 8), diabetic glomerulopathy (n = 12), polycystic kidney disease (n = 3), nephrosclerosis (n = 3), focal segmental glomerulosclerosis (n = 10), IgA nephropathy (n = 8), membranous glomerulonephritis (n = 11), mesangiocapillary glomerulonephritis (n = 1), and unknown (n = 4). Immunosuppressive regimen included steroids (methylprednisolone at the initial dose of 500 mg, followed by 1 mg · kg-1 · day-1, progressively tapered) and cyclosporine A (initial dose of 8 mg · kg-1 · day-1, and then adjusted according to blood concentrations) or FK506 in cases of cyclosporine intolerance (at the dose of 0.1 mg · kg-1 · day-1). The patients were classified indo two groups according to the clinical diagnosis: with or without delayed graft function.

The control group consisted of 50 healthy persons. They were free of cardiac, liver or renal diseases or hypertension and had normal urine analysis and normal sera urea,creatinine and Cys C concentration.

Methods

Delayed graft function (DGF) was defined as a requirement for dialysis during the first 2 weeks after transplantation. All patients were on conventional dialysis. Episodes of acute rejection diagnosed by renal biopsy were treated with 5 days of intravenous methylprednisolone. This study was in accordance with the ethics standards of the Helsinki Declaration of 1975, revised in 1983.

Allograft function was evaluated on a daily basis starting on the day of surgery (day 0) and for 25 days thereafter or until hospital discharge, whichever occurred first. Blood (7 mL) was drawn by venipuncture in a Vacutainer® Tube (Becton Dickinson) before centrifugation (3500g at 20 °C for 15 min) and analyzed for creatinine and CysC. Creatinine concentration was enzymatically assayed on a INTEGRA biochemical analyzer. The sample volume was 10 µL, and the assay was performed at 37°C; total analysis time was 7 min. The interassay imprecision (CV; n = 50) was <3%.

The concentration of CysC was measured using a DAKO Immunoturbidimetric assay on a Cobas Mira biochemical analyser.The reference serum interval was 0.80 – 1.25 mg/L. Briefly, the assay is performed at room temperature with a six-point calibration covering the range of 0.23–7.25 mg/L. The calibrator used is a purified Cys C from human urine (1.45 mg/L). The sample volume is 80 µL. The time for analysis is 6 min, each subsequent sample reading being available after 8 s. The interassay CV (n = 20) was <4% for both the low (1.4 mg/L) and high (4.2 mg/L) controls.

Calculations

The percentage of discordant changes in plasma cystatin C and creatinine concentrations was calculated on a day-to-day basis after transplantation. A change in opposite direction (increase/decrease) of >10% between the two markers was considered as discordant. On hospital discharge or at the end of the 25 days period, creatinine clearance was estimated from plasma creatinine using the formula of Cockcroft and Gault (14). A cutoff of 80 mL/min was selected for normal estimated creatinine clearance (25).

Statistical analysis

Data are presented as mean ± SD or as median and range when appropriate after checking for gaussian distribution. Differences between two groups were evaluated

by the Wilcoxon's signed-rank test. Multiple comparisons were performed by the Friedman's repeated-measure ANOVA on ranks followed by the Dunn's test. Correlation between techniques was evaluated by linear regression and ANOVA. Results with P <0.05 were considered statistically significant.

Results and discussion

CysC and creatinine concentration were measured in 50 healthy adults (39 +/- 9 years). Data were analyzed by linear regression and ANOVA (P < 0.05 considered significant).The concentration of Cystatin C significantly correlated (r = 0.625; P<0.001) with creatinine (Fig. 1). Cystatin C serum concentration was not influenced by age (r=0.195; not significant). The calculated reference interval for creatinine was 58-109 mmol/L (males and females) and the cystatin C : 0.80 – 1.25 mg/L.

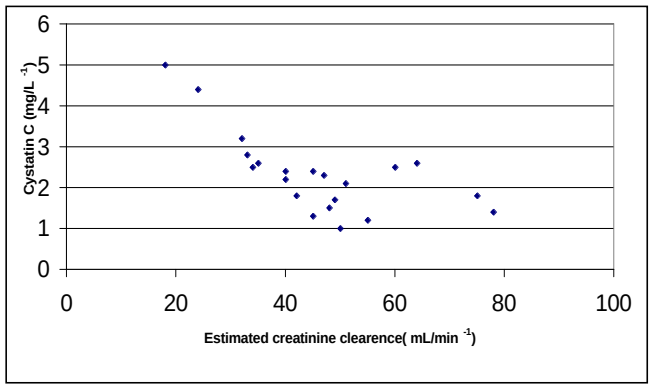

Fig. 1. Relationship between serum Cystatin C and serum creatinine in healthy controls (r= 0.625, p < 0.001)

Transplant patients were separated into two groups: normal course (absent of complications; n= 38) (Fig. 2); DGF and acute rejection episode defined as requiring hemodialysis during the first 2 weeks after surgery (n=26).

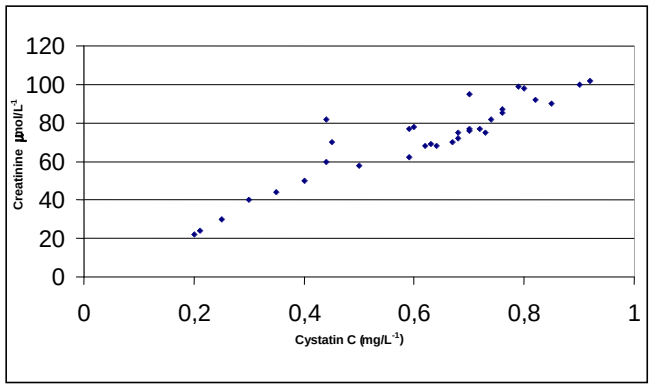

Fig. 2. Serum Cystatin C and serum creatinine in renal transplant patients without DGF

In patients without DGF a significant decline in plasma concentration was more rapidly obtained for cystatin C that for creatinine.

Starting on day 4 post-transplantation the decrease in plasma concentration in patients without DGF was more pronounced for creatinine that for cystatin C (Fig. 3).

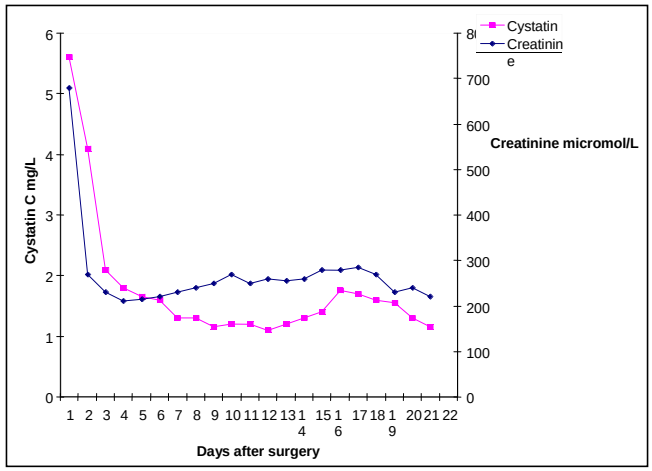

Fig. 3. Serum Cystatin C and serum creatinine in renal transplant patients with DGF

In patients with DGF, the reduction in plasma concentration was not significant until day 14 for creatnine and day 17 for cystatin C.

At the end of the 25 days - study period, a 50% reduction from the initial plasma creatinine concentration was observed in patients with DGF vs 35% for Cystatin C.The frequency of discordant daily changes in cystatin C and creatinine values was significantly higher in patients with DGF(40%) than in those without DGF (21%,P < 0.01).

In cases with acute rejection episode was demonstrated by persistent increases in both plasma creatinine and cystatin C concentration. Serum cystatin C gradually increase (115%) during 7 days before diagnosis (nephrotoxicity – FK506-regressed spontaneously).

At the end of the study (day 25), serum creatinine was 150 µmol/L (79–602 µmol/L) vs 2.12 mg/L (1.04–5.54 mg/L) for cystatin C. Both markers significantly correlated (r = 0.812; P <0.001). Estimated creatinine clearance by the Cockcroft and Gault formula (14) in patients with stable renal function (no hemodialysis during the last week, n = 19) was 49 mL/min (15–66 mL/min), and no patient was within the reference interval (>80 mL/min). Serum creatinine was within the reference interval for three patients (80-109 µmol/L) compared with none for cystatin C. The relationship between estimated creatinine clearance and the serum concentrations of creatinine and cystatin C in patients with stable renal function (n = 22) is presented in Fig. 4 and 5.

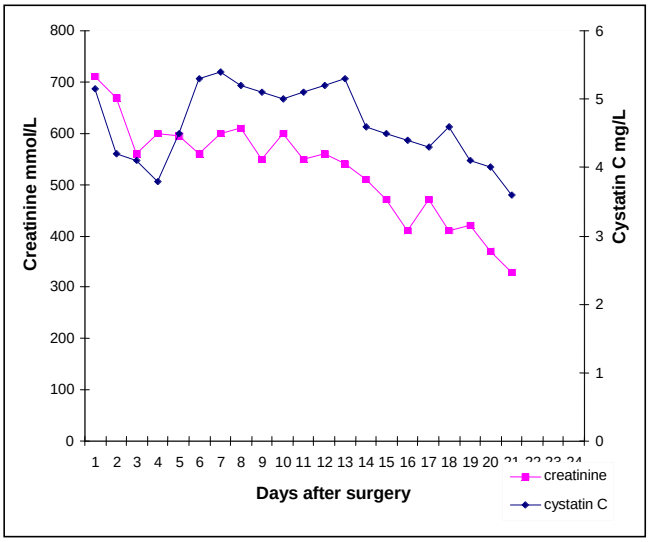

Fig. 4. Relationship between serum Cystatin C and GFR

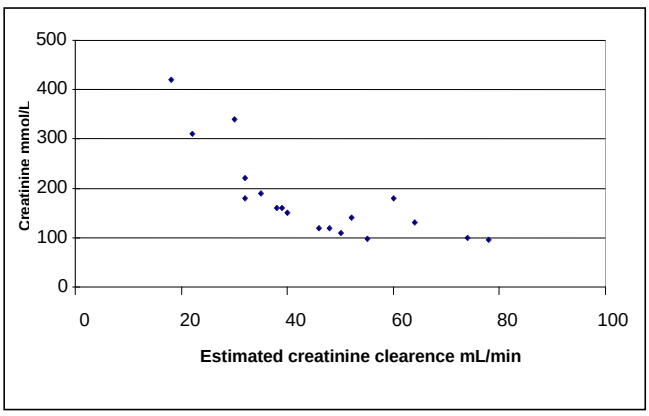

Fig. 5. Relationship between serum creatinine and GFR

There was a significant correlation between estimated creatinine clearance and serum cystatin C ($r = 0.714$; $P < 0.001$).

Discusion

Sensitive and reliable recognition of changes in GFR is of primary importance in transplant patients. A DGF is an identified and independent risk factor for graft survival (26) Acute rejection is also an established risk factor for renal graft failure, defined as a return of patient to hemodialysis (18-20). Recent studies have suggested that cystatin C might be a potential better marker of GFR than plasma creatinine such as in renal transplant patients (28-30).

The mean cystatin C plasma concentration in our group of healthy adults was similar to that reported in a group of healthy subjects (n = 50; mean age, 40 years), using an immunoturbidimetric assay (0.65 ± 0.05 mg/L) (30). Slightly higher values (mean cystatin C, 0.80 mg/L) were reported by others (27) in a group of 52 adults with normal renal function (ages, 21–79 years). These data are in agreement with an increase in plasma cystatin C values with age, especially after the age of 50 years (35). We found no the sex difference reported previously (22,23). Serum cystatin C correlated poorly with serum creatinine, as reported previously in subjects with GFR >80 mL/min (24) or GFR >70 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (23). These data suggest that different physiological factors (such as sex, dietary factors, or body composition) influence cystatin C and creatinine plasma concentrations in healthy adults.

In renal transplant patients, plasma cystatin C concentrations paralleled those of creatinine regardless of graft function (absence or presence of DGF). Consequently, serum cystatin C and creatinine significantly correlated over the postoperative study period as observed previously in adult renal transplant patients (31) and subjects suffering from chronic renal disease (13). Some differences, however, were apparent in their respective plasma kinetics. During the first 4 days post-transplantation, the cystatin C serum concentration decreased more rapidly than that of creatinine. Tanstubular leakage of the low-molecular weight (M_r 100) creatinine (23) has been reported in acute renal failure, thus leading to high plasma creatinine values. Starting on day 4 post-transplantation, decrease in serum concentration became more prominent for creatinine than for cystatin C, which might be attributable to an underestimation of GFR by plasma cystatin C, an overestimation of GFR by plasma creatinine, or both. A stronger correlation between cystatin C than between creatinine and the measured GFR has been reported in adults suffering from renal diseases (18).

For patient follow-up, the ability to detect rapid changes in GFR is clinically more important than accuracy itself. With diminished GFR, a significant increase in plasma concentration of cystatin C and creatinine will depend on the rate of its accumulation in plasma, which depends on its production rate and distribution volume, but also on its biological intraindividual variation. Repeated measures obtained in healthy subjects (30) suggested that intraindividual variation might be more important for cystatin C (13.3%) than for creatinine (4.9%). If true, cystatin C would be less sensitive for the detection of acute rejection episodes for a given individual than creatinine (30). In all episodes of acute rejection and acute nephrotoxicity in our study, the plasma cystatin C concentration broadly paralleled that of creatinine. The rise in plasma cystatin C concentration was more prominent than in creatinine.

After renal transplantation, hemodialysis was required in almost 55% of our patients. We found a higher degree of discrepancy (45% of discordant results; $P < 0.01$) between cystatin C and creatinine kinetics in patients requiring hemodialysis than in those with a normal course (19%). In addi-

tion, cystatin C and creatinine weakly correlated in hemodialyzed patients (r = 0.429). The molecular weight of cystatin C is 13 300 with an Einstein-Stokes radius of 30–40 Å, which is much higher than creatinine (M_r 100 and 3 Å) (15). In a large study of 112 patients on stable maintenance hemodialysis, a 30% reduction in serum cystatin C was observed after dialysis with mostly AN69 high-flux membranes (23). As expected, the elimination of cystatin C during dialysis increased with the ultrafiltration coefficient (UFC) of the membrane, an estimate of the permeability: 0% (vs 40% for creatinine) for UFC <15 mL $\cdot$ h^{-1} $\cdot$ m^2 $\cdot$ mmHg and ~ 60% (as for creatinine) for UFC >15 mL $\cdot$ h^{-1} $\cdot$ m^2 $\cdot$ mmHg (23). Thus, removal of cystatin C by hemodialysis seems highly dependent on the type of membrane selected. This is an important issue in renal transplantation because it could limit the use of plasma cystatin C as a marker of graft function in patients with DGF. On the other hand, if poorly filtered by dialysis membranes, plasma cystatin C could be used by the nephrologist to monitor appropriate duration of hemodialysis in patients with DGF. Prolonged unnecessary hemodialysis could be avoided as soon as a significant decrease in plasma cystatin C is obtained by dialysis.

In conclusion, the determination of cystatin C concentration is an alternative and more accurate marker of GFR than creatinine in adult transplantation. In some cases, a more prominent rise in serum cystatin C values allows a more rapid diagnosis of acute rejection or treatment nephrotoxicity. Further prospective studies are needed to evaluate this last issue and the potential of plasma cystatin C in the long term follow-up of graft function in renal transplantation.

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Резиме

Серум Cistatin C кај пациенти со нарушена графт функција

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Клучни зборови: ренална трансплантација, нарушена графт функција, Cystatin C

Изработената студија укажува дека серумската концентрација на Cystatin C (Cys C) е подобар маркер за GFR во споредба со стандардниот креатинин, што беше и клинички евалиурано. Овој маркер е многу поосетлив за графт функцијата после бубрежна трансплантација. Концентрацијата на Cys C е споредена со онаа на креатининот. Намалувањето на бубрежната функција е следено кај 64 трансплантирани пациенти. Концентрацијата на Cys C сигнификантно корелира (r=0.625, p<0.001) со креатининот кај контролната група. Кај овие пациенти средните креатинин и цистатин Ц концентрации се: 81+/-13 mmol/L односно 0.90 +/-0.22 mg/L. Плазма концентрациите на цистатин Ц и креатининот сигнификантно корелираат во пост-трансплантациониот период (r=0.686, p<0.001), но потврдивме разлики во кинетиката на овие параметри. Во првите четири дена после трансплантација Cys C се нормализира побрзо од таа на креатининот. Развитокот на акутна реакција (помеѓу 5 и 7 ден) укажува на високата осетливост и специфичност на промените на Cys C споредени со тие на креатининот.