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Is Serum Cystatin C a Sensitive Marker of Glomerular Filtration Rate (GFR)? A Preliminary Study on Renal Transplant Patients

Mario Plebani,[1,2] MD, Roberto Dall'Amico,[3] MD, Michele Mussap,[1] MD, Giovanni Montini,[3] MD, Nicoletta Ruzzante,[1] MD, Renzo Marsilio,[3] PhD, Giuseppe Giordano,[3] PhD, and Graziella Zacchello,[3] MD

[1]*Department of Laboratory Medicine*
Central Laboratory
Azienda Ospedaliera
Padova, Italy
[2]*Center of Biomedical Research*
Veneto Region
Castelfranco Veneto (TV), Italy
[3]*Department of Pediatric*
School of Medicine
University of Padova
Padova, Italy

ABSTRACT

Human cystatin C is a basic low molecular mass protein (13,359 Dalton) freely filtered through the glomerulus and almost completely reabsorbed and catabolized by proximal tubular cells. We measured serum cystatin C in 38 kidney transplant patients (23 males, 15 females) aged between 6 and 32 years. To assess renal function, serum and urinary creatinine were also determined in all patients, and creatinine clearance was finally calculated. Cystatin C was determined by a particle-enhanced turbidimetric assay, and creatinine was measured by gas

Address correspondence to: Mario Plebani, MD Department of Laboratory Medicine, Central Laboratory, Azienda ospedaliera Padova, via N. Giustiniani, 2-35128 Padova (Italy). Tel.++ 39 49 8212792; Fax: ++39 49 663240.

chromatography-mass spectrometry. To compare the diagnostic efficiency of cystatin C with that of creatinine, inulin clearance was performed on 12 renal transplant patients, and receiver operating characteristic (ROC) analysis was applied. The results of this study demonstrate that serum cystatin C significantly increases in renal transplant patients with reduced creatinine clearance (< 70 mL/min per 1.73 m^2) and that the diagnostic accuracy of serum cystatin C is better than of serum creatinine. Cystatin C may be utilized as a very marker of reduced GFR.

Key words: Glomerular filtration rate (GFR); Low molecular mass proteins; Cystatin C; Renal transplant patients.

INTRODUCTION

The estimation of glomerular filtration rate (GFR) is commonly performed by measuring renal clearance of exogenous or endogenous substances and by determining endogenous serum markers. Unfortunately, the ideal marker for measuring GFR has yet to be discovered. Although inulin clearance is considered the "gold standard test", it requires specialized technical personnel over a period of several hours (1). Serum creatinine and creatinine clearance are the most widely used methods for the noninvasive estimation of GFR in clinical practice. Serum creatinine is considered relatively specific, but not very sensitive since its levels only increase significantly when more than 50% of the GFR is reduced. In the past, it was postulated that serum levels of low molecular mass proteins (2), such as β_2-microglobulin (3), α_1-microglobulin (4), retinol binding protein (5), etc., might reflect changes in GFR, because these proteins are freely filtered by the glomerulus and then almost completely reabsorbed and catabolized by proximal tubular cells (6). However, no protein marker has been effectively introduced into clinical practice, perhaps because they are significantly influenced by several extra-renal factors. Recently, cystatin C (13,359 Dalton) has been proposed as a new sensitive and accurate endogenous marker of changes in GFR (7,8). Human cystatin C is a basic low molecular mass protein with 120 amino acid residues, steadily produced by all human nucleated cells studied. It Is freely filtered through the glomerulus and almost completely reabsorbed and catabolized by proximal tubular cells. The stable production rate of cystatin C, its low molecular mass and Its renal catabolism have suggested that the major determinant of serum cystatin C concentration is GFR (9). In addition, this protein is not significantly influenced by inflammation or by malignancy (10). We tested the diagnostic value of cystatin C as a serum marker of changes in GFR in a group of renal transplant patients with normal to markedly reduced creatinine clearance. To compare the diagnostic accuracy of serum cystatin C and that of creatinine in distinguishing between normal and reduced GFR, inulin clearance was also performed in 12 of these renal transplant patients.

PATIENTS AND METHODS

Thirty-eight renal transplant patients (23 males and 15 females), aged between 6 and 32 years were studied. Their body weight ranged from 13.6 to 77.5 Kg and their body

surface from 0.7 to 1.92 m^2. In all patients we determined serum cystatin C, creatinine, and creatinine clearance. On the basis of their creatinine clearance values normalized for body surface, patients were divided into two groups: group A (n = 22) with a creatinine clearance < 70mL/min per 1.73 m^2, and group B (n = 16) with creatinine clearance > 70mL/min per 1.73 m^2. In addition, to assess the diagnostic efficiency of serum cystatin C in comparison to that of serum creatinine in predicting changes in GFR, we performed inulin clearance on 12 patients. Cystatin C was measured by an automated particle-enhanced immunoturbidimetric assay (PET, Dako Co., Milano, Italy) (7). Creatinine was measured by isotope dilution gas chromatography-mass spectrometry (GC–MS), in according to the reference method (11). Inulin was measured by a reversed-phase HPLC method (12). Data were evaluated by standard parametric tests, using StatView SE + Graphics™ statistical software (Abacus Concepts Inc., Berkeley, CA, USA). Differences in variables between groups were analyzed by one-way analysis of variance (ANOVA), followed by the Sheffé multiple comparison test; $p < 0.05$ was considered statistically significant. The relationships between variables were investigated by using simple linear regression. We constructed receiver-operating characteristic (ROC) plots in order to compare the diagnostic efficiency of cystatin C and that of creatinine. ROC curves were generated by plotting the sensitivity vs 1-specificity, giving the ideal test a sensitivity equal to 1 and a specificity equal to 1 (corresponding to 1-specificity equal to zero). The area under the curve (AUC) represents the most common overable measure of the diagnostic efficiency of a test. Traditionally, this AUC is always > 0.5, values ranging from 1 (ideal perfect separation of the test values between groups of patients and controls) to 0.5 (no apparent distribution difference between the two groups). In according to Hanley and McNeil (13), we calculated areas under the curves, 95% confidence intervals (14), and differences between ROC curves. A value > 1.96 of the critical ratio z, defined as z-score, was considered significant, as indicated elsewhere (15).

RESULTS

In all transplant patients no correlation was found between serum cystatin C levels and weight, body surface, and body mass index (BMI). In group A (creatinine clearance < 70 mL/min per 1.73 m^2) serum cystatin C ranged from 0.85 to 4.33 mg/L (median 2.1) and serum creatinine from 90 to 426 μmol/L (median 196). In group B (creatinine clearance > 70 mL/min per 1.73 m^2) serum cystatin C ranged from 0.41 to 1.71 mg/L (median 1.24) and serum creatinine from 64 to 99 μmol/L (median 78). ANOVA showed a statistically significant difference between serum cystatin C values in group A and those of group B ($p < 0.0001$). In group A, serum cystatin C (y) and creatinine (x) correlated significantly ($y = 0.16x + 0.101$, $r = 0.815$, $Sy/x = 0.737$, $p < 0.0001$), while in group B no correlation between these two markers was found ($y = 0.01x + 0.796$, $r = 0.157$, $Sy/x = 0.52$, p = n.s.) (Figure 1). By plotting reciprocal values of serum cystatin C (y) versus GFR, as determined by the clearance (x), simple linear regression showed a significant relationship between these two variables ($y = 0.003x + 0.487$, $r = 0.67$, $Sy/x = 0.325$, $p < 0.0001$) (Figure 2). ROC analysis demonstrated a significant difference between the diagnostic efficiency of cystatin C and that of creatinine for the assessment of changes in GFR (Figure 3). In fact, the area under the cystatin C ROC curve (0.859;

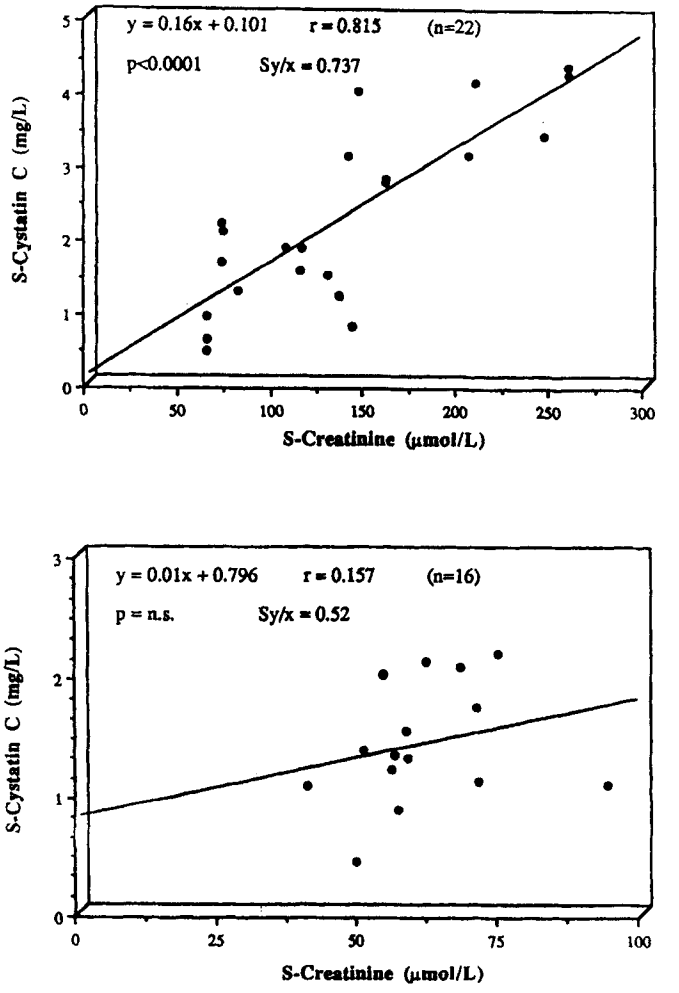

Figure 1. Simple linear regression between serum creatinine and cystatin C in renal transplant patients with creatinine clearance < 70mL/min/1.73m^2 (top) and in patients with creatinine clearance > 70mL/min/1.73m^2 (bottom)

95% confidence interval 0.727–0.99) significantly differs (z-score = 18.1) from that under creatinine ROC curve (0.743; 95% confidence interval 0.573–0.913).

DISCUSSION

This preliminary study on cystatin C in renal transplant patients seems to indicate that this serum marker is not significantly influenced by extra-renal factors, such as weight, body surface, and BMI. In this study we used a definitive method to assess serum creatinine levels, in order to minimize analytical interferences. In fact, substances other

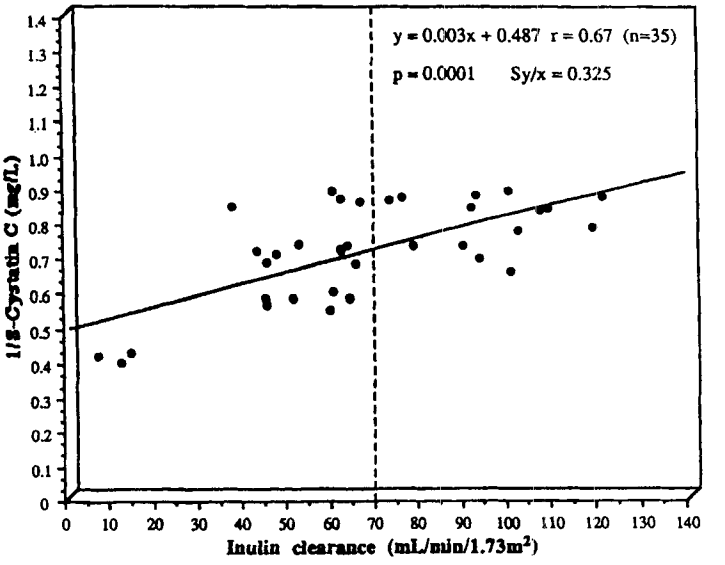

Figure 2. Relationship between GFR (measured by inulin clearance) and reciprocal values of serum cystatin C

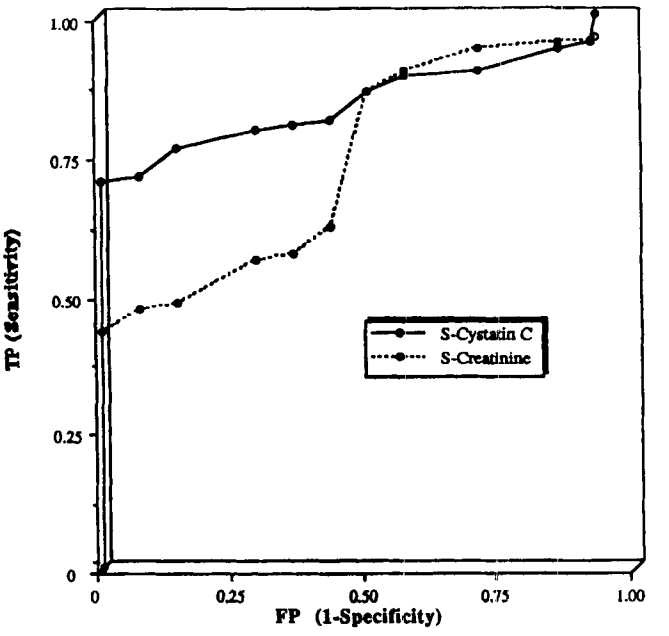

Figure 3. ROC curves for serum cystatin C and serum creatinine in renal transplant patients.

than creatinine, called noncreatinine chromogens, may positively or negatively up to 20% interfere on routine analytical assays for creatinine measurement (standard or modified Jaffe reaction and enzymatic methods). By using the definitive method, endogenous compounds, such as glucose, acetoacetate, ascorbate, bilirubin, as well as esogenous substances, such as cyclosporin, certain cephalosporins, etc. do not influence the analytical measurement. In patients with creatinine clearance > 70mL/min per 1.73 m^2, the mean cystatin C concentration was found to be comparable with that previously observed in a group of healthy adults, using the same analytical assay. Our results evidence that cystatin C is closely related to GFR, significantly increasing when inulin clearance values decrease, as well as the well-known relationship between inulin clearance and serum creatinine. This significant relationship is largely accounted for by data from patients with reduced renal function, as showed in figure 2. Although cystatin C and creatinine correlate well each other in patients with creatinine clearance <70mL/min per 1.73 m^2, in patients with creatinine clearance > 70mL/min per 1.73 m^2 no correlation between these two indexes was found. Thus, they may be considered two independent serum markers of changes in GFR. The diagnostic accuracy of cystatin C, assessed by ROC analysis, was greater than that of creatinine in discriminating between patients with normal GFR and those with reduced GFR. Thus, when the object is to identify subjects with GFR impairment (sensitivity), serum cystatin C may be clinically utilized as a more efficient diagnostic tool than serum creatinine. However, further studies are required in order to confirm these findings on more representative numbers of patients.

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