Transpl Int (2000) 13: 285–289

ORIGINAL ARTICLE

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Cystatin C – an accurate marker of glomerular filtration rate after renal transplantation?

Received: 5 June 1999
Revised: 8 March 2000
Accepted: 20 April 2000

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Abstract The performance of serum cystatin C as a screening marker of reduced creatinine clearance in renal transplantation was evaluated and compared to serum creatinine. In addition we studied whether cystatin C accurately reflects creatinine clearance over the entire range of transplant function. Serum cystatin C, serum creatinine, and creatinine clearance were measured in 110 adult renal transplant recipients. Cystatin C detected reduced creatinine clearance with the high sensitivity of 95 %. Serum cystatin C and serum creatinine did not differ regarding 90 and 95 % sensitivity, derived from the receiver-operating characteristics plot. We demonstrated a strong correlation and linear association between 1/cystatin C and creatinine clearance over the entire range of transplant function, equivalent to that of 1/creatinine. In summary, serum cystatin C accurately reflects creatinine clearance over the entire range of transplant function and is as efficacious as serum creatinine to detect reduced creatinine clearance in renal transplant recipients.

Key words Cystatin C · Diagnostic test · Glomerular filtration rate · Kidney transplantation · ROC curve · Sensitivity

Introduction

In renal transplantation, the detection and monitoring of transplant dysfunction is of great importance. Glomerular filtration rate (GFR) is considered to be the best marker of renal transplant function. The measurement of serum creatinine is the standard method for rapid estimation of GFR. However, this method has disadvantages. In patients with transplant dysfunction, GFR can halve before creatinine increases [11, 22]. A mild elevation of creatinine, as typically seen in renal transplant recipients, may already reflect significant transplant dysfunction [21], and further deterioration of transplant function may not be accurately reflected by slowly rising serum creatinine [12]. In addition, creatinine may inaccurately estimate GFR in renal transplant recipients due to decreased muscle mass caused by glucocorticoid administration, increased tubular secretion of creatinine, or during rejection episodes [9, 11, 13, 22].

Serum cystatin C has been proposed in patients without transplantations as a screening test of reduced GFR [4, 5, 6, 8, 14, 23, 24]. Cystatin C is a cysteine proteinase inhibitor, which is produced by all nucleated cells at a constant rate [1]. It is freely filtered by the glomerulus, reabsorbed and catabolized in the tubules [25]. Based on these characteristics, cystatin C seems well suited as a marker of GFR. Previous studies assessing serum cystatin C in patients without transplantations, found it su-

perior to serum creatinine detecting GFR reduction [4, 5, 6, 8, 14, 24]. Two studies have been published testing renal transplant recipients [15, 19]. Both demonstrated a higher diagnostic efficiency for cystatin C than for creatinine. However, these studies comprised only small cohorts, one only young renal transplant recipients, and indices of cystatin C's accuracy as sensitivity or specificity were not reported [15].

The purpose of this study was to evaluate serum cystatin C as a screening marker of reduced GFR in comparison to serum creatinine for the first time in a large cohort of non-selected, adult renal transplant recipients representing all different ages. In addition, we assessed the association of cystatin C with creatinine clearance to determine whether cystatin C accurately reflects creatinine clearance over the entire range of transplant function.

Patients and methods

Patients

One hundred-ten consecutive renal transplant recipients (53 female, 57 male), age 49 ± 14 years (19–74 years), undergoing renal function testing at the University Hospital Essen, were prospectively studied. Informed consent was obtained from all patients prior to enrollment, and the study was performed in accordance with the ethical standards set down in the 1964 declaration of Helsinki. The patients underwent transplantation 58 ± 57 (2–265) months prior to the study and underwent the following immunosuppressive maintenance regimes: cyclosporin A and prednisone (n = 49); cyclosporin A, azathioprine and prednisone (n = 18); cyclosporin A, mycophenolat mofetil and prednisone (n = 9); azathioprine and prednisone (n = 7); tacrolimus and prednisone (n = 14); tacrolimus, azathioprine and prednisone (n = 6); tacrolimus, mycophenolat mofetil and prednisone (n = 7). In the 3 weeks after the renal function testing, renal transplant biopsies were performed in 31 patients. They demonstrated chronic rejection (n = 16), acute rejection (n = 5), interstitial fibrosis (n = 4), glomerulonephritis of the transplant (n = 5), or cyclosporin A nephrotoxicity (n = 1).

Methods

Serum concentrations of cystatin C and creatinine were determined from the same blood sample, which was obtained on the day of the creatinine clearance measurement. Serum cystatin C concentration was measured with a particle enhanced immunonephelometry (Dade Behring, Marburg, FRG), using a BNA type nephelometer (Behring). The reference range for cystatin C, previously determined by us in 200 healthy blood donors, was 0.45 to 1.01 mg/l [6]. Serum and urine creatinine concentration were measured by a modified Jaffe method with protein precipitation using an alkaline pictrate reaction. Upper reference values of creatinine were 1.0 mg/dl for females, 1.3 mg/dl for males younger, – and 1.2 mg/dl for males older than 50 years. A two by two-hour creatinine clearance, which has been demonstrated to be accurate and reproducible [7], was performed as follows: 2 consecutive 2-h urine collections were performed in fasting patients between 06.00 and 10.00 a.m. with 100 ml fluid intake per hour. Two separate 2-h creatinine clearance values were calculated, corrected for 1.73 m^2 body surface, and the means taken for further analysis.

Statistics

Sensitivity, specificity, positive and negative predictive value of serum cystatin C and serum creatinine indicating reduced creatinine clearance were calculated according to age- and gender specific reference values of creatinine clearance as determined in [10]. Receiver-operating characteristic (ROC) plots simultaneously showing sensitivity and specificity for cystatin C and creatinine were generated. At the points of 90% and 95% sensitivity and specificity, false negatives of cystatin C and creatinine were compared using a two-sided Mc-Nemar's test. As serum creatinine is inversely related to creatinine clearance [11], the reciprocals of serum cystatin C and serum creatinine were assessed for association with creatinine clearance. The correlation coefficients were compared using the Normal test after Fisher's Z-transformation. The first principal component was drawn into the scatter diagrams to illustrate the linear association. A P value of less than 0.05 was considered to be statistically significant. Data are presented as mean ± SD with ranges in parenthesis, unless stated otherwise. Statistical analyses were performed with the SAS program version 6.12 (SAS Institute, Cary, N.C., USA).

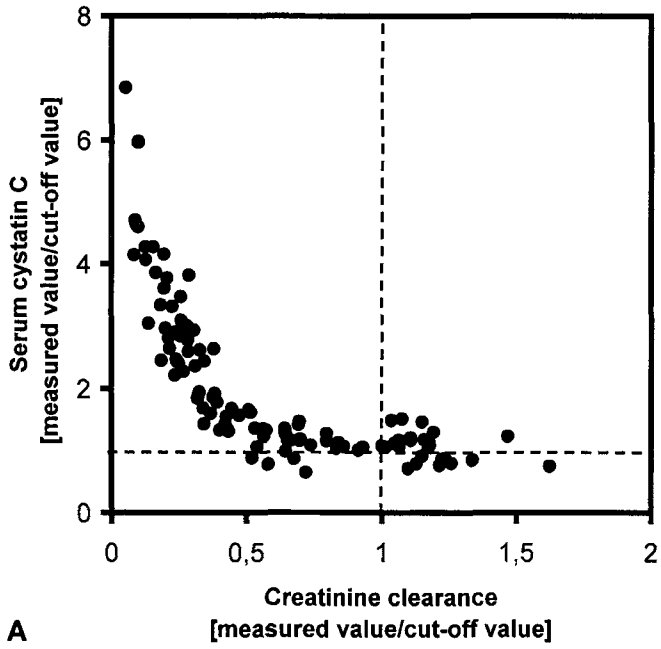
Results

The mean value for serum cystatin C was 2.33 ± 1.09 mg/l (0.76–6.92) with a coefficient of variation of 0.51, for serum creatinine 2.06 ± 1.05 mg/dl (0.92–7.51) with a coefficient of variation of 0.47, and for 2 × 2 h creatinine clearance 43 ± 22 ml/min/1.73 m^2 (5–111) in the 110 renal transplant recipients studied. 76.4% of the patients in our study had a reduced GFR as measured by creatinine clearance. As shown in Table 1, the patients were distributed over the entire range of renal function.

Serum cystatin C demonstrated a high overall sensitivity of 95%, detecting reduced creatinine clearance, but low overall specificity of 31%, serum creatinine, a sensitivity of 83% and a specificity of 67%. Figure 1A and B, showing the relationship between cystatin or creatinine and creatinine clearance, all expressed as ratio

Table 1 Distribution of patients, mean serum cystatin C and serum creatinine concentration according of creatinine clearance classification

Creatinine Clearance (ml/min/ 1.73m^2)	0–20	21–40	41–60	61–80	> 80
n	8	24	36	29	13
Cystatin C (mg/l)	4.8 ± 1.1	3.3 ± 0.7	2.1 ± 0.4	1.6 ± 0.3	1.3 ± 0.2
Creatinine (mg/dl)	4.5 ± 1.3	2.9 ± 0.6	1.8 ± 0.4	1.5 ± 0.3	1.2 ± 0.2



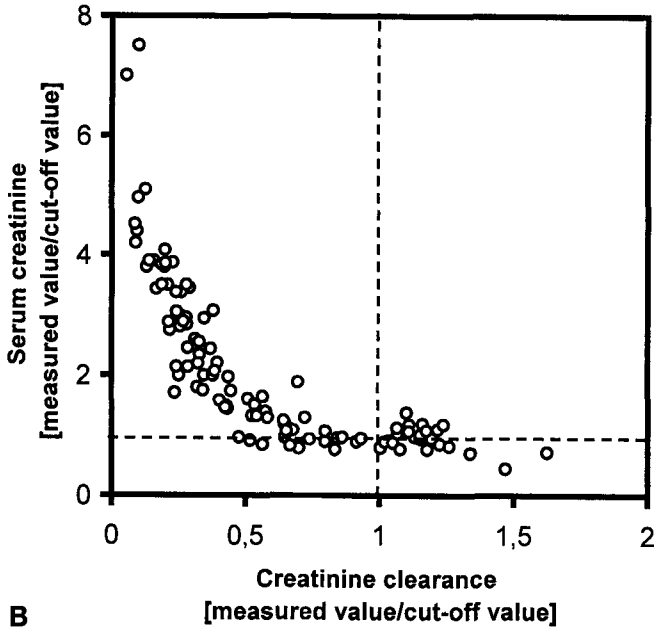


Fig. 1 Relationship between serum cystatin and creatinine clearance **A**, and serum creatinine and creatinine clearance **B**. Values for cystatin C, creatinine and creatinine clearance are expressed as ratio of measured value to cut-off value. The cut-off values applied are described in Patients and methods. The dashed lines represent the cut-off values for serum cystatin C, serum creatinine and creatinine clearance respectively

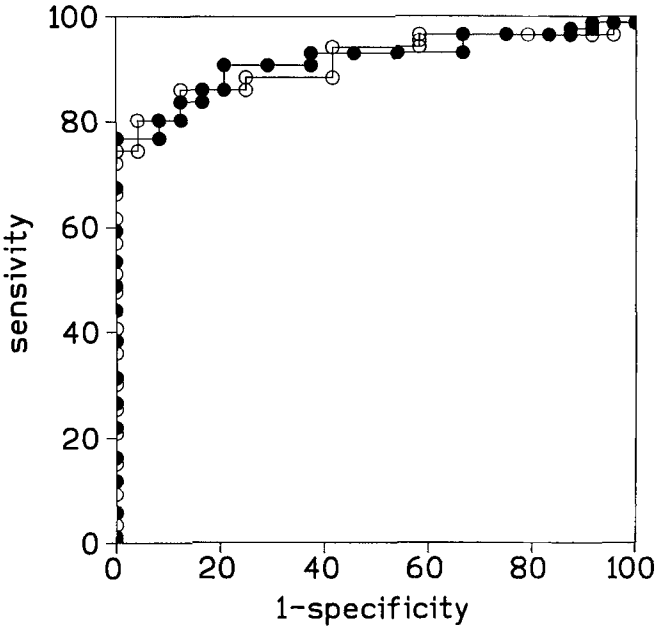


Fig. 2 Nonparametric ROC plots of sensitivity and specificity of serum cystain C (●) and serum creatinine (○) in percent, discriminating between normal and reduced creatinine clearance. Reference values of creatinine clearance as determined in [11]

of measured value to cut-off, illustrate the diagnostic value of both diagnostic tests. ROC plots, allowing to evaluate the performance of cystatin C and creatinine over the whole spectrum of test results without limit to predefined cut-off values, confirmed the high sensitivity for both cystatin C and creatinine (Fig. 2). As Fig. 2 demonstrates, ROC curves of both tests were similar, indicating no marked difference in sensitivity or specificity detecting reduced creatinine clearance. In addition, serum cystatin C and serum creatinine did not significantly differ with regard to 90 and 95% sensitivity, as well as 90 and 95% specificity, derived from the ROC plot.

There was no linear, but a hyperbolic dependency between serum cystatin C and creatinine clearance, equivalent to the correlation known for serum creatinine and creatinine clearance (Table 1). Significant correlation was found for 1/cystatin C versus creatinine clearance ($r = 0.87$; $P < 0.001$). This was similar to the correlation of 1/creatinine versus creatinine clearance ($r = 0.85$; $P < 0.001$). Both correlation coefficients for 1/cystatin C and for 1/creatinine versus creatinine clearance were equivalent, and their difference was not significant. The association of 1/cystatin C and of 1/creatinine with creatinine clearance was expressed as the first principal component (Fig. 3A and B). Concluding from Fig. 3A and B, linear association is most probable between 1/cystatin C and creatinine clearance, as previously demonstrated for 1/creatinine [11]. There was little scat-

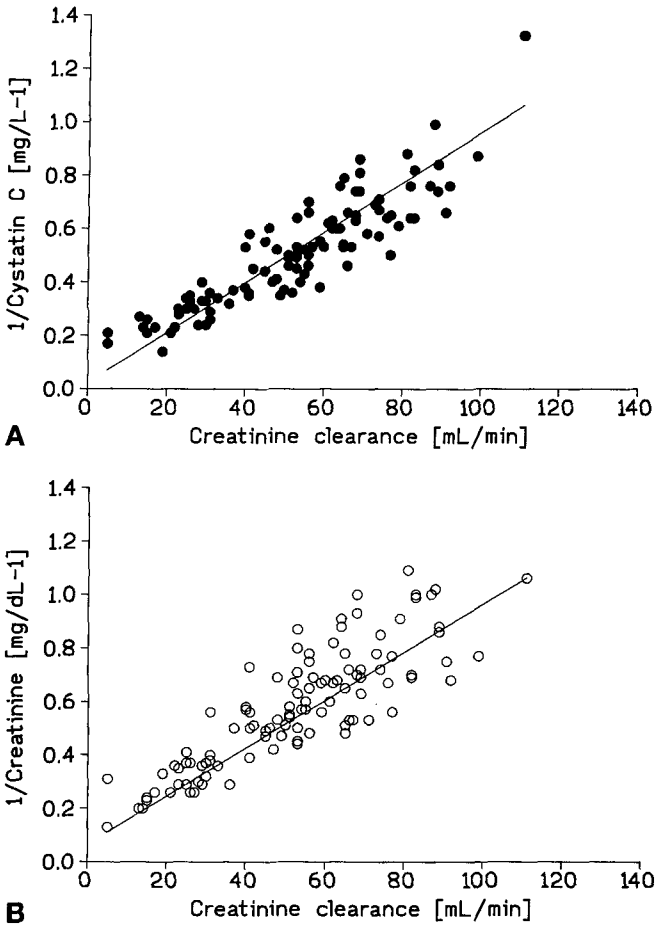


Fig. 3 Correlation of 1/cystatin C and creatinine clearance **A**, and of 1/creatinine and creatinine clearance **B**. The association between serum cystatin C, and serum creatinine respectively versus creatinine clearance is illustrated by the principal component *(straight lines)*

ter present in the plot of 1/cystatin C versus creatinine clearance. This scatter is less marked than in the plot of 1/creatinine versus creatinine clearance.

Discussion

This study shows, that serum cystatin C is as efficacious as a screening marker for detecting renal transplant dysfunction as serum creatinine. Serum cystatin C yields a high sensitivity, discriminating between normal and reduced creatinine clearance. Although cystatin C has a higher overall sensitivity than creatinine, 95% and 90% sensitivity derived from ROC plots are equivalent in both markers. The high sensitivity of serum cystatin C as a marker for detecting reduced creatinine clearance is in accordance with previous studies in patients who did not undergo transplantation [4, 5, 6, 8, 14, 24]. In these studies however, cystatin C performs better than creatinine. The incongruous results may be explained by several factors: Our cohort consisted of adult renal transplant recipients, others of children [4, 5, 23], or of adults who did not undergo transplantation [8, 14]. The 76.4% prevalence of renal dysfunction in our cohort is higher than in previous studies. Possible alterations in glomerular or tubular handling of cystatin C, due to the high rate of renal transplant dysfunction, may contribute to the differences in performance of serum cystatin C.

According to our data, there is a linear association between 1/cystatin C and creatinine clearance. This result is important, as it demonstrates that 1/cystatin C accurately reflects creatinine clearance over the entire range of renal transplant function. Although the correlation between 1/cystatin C and creatinine clearance, as measured by correlation coefficient, is superior to the correlation coefficient between 1/creatinine and creatinine clearance, the small difference is not significant. This does not correspond with previous studies, as these studies reported the correlation between 1/cystatin C and GFR to be significantly higher than that of 1/creatinine and GFR [4, 5, 8, 14, 15, 24].

Our results indicate a hyperbolic relationship between serum cystatin C and creatinine clearance as it is known for serum creatinine and creatinine clearance. This hyperbolic relationship implies that at a GFR close to normal, large changes in GFR correspond with only small changes in cystatin C. Therefore, mild elevation of serum cystatin C as typically seen in renal transplant recipients, may already reflect marked impairment of renal function, leading to overestimation of GFR. Using creatinine clearance as reference method to assess the performance of serum cystatin C and serum creatinine, we are aware that creatinine clearance can result in an inaccurate estimate of GFR [2, 22, 26]. However, good agreement between creatinine clearance and "true" markers of GFR as inulin [3], ^{125}I-iothalamate [16] and ^{99m}Tc-DTPA clearance [17, 20] at all levels of renal function was reported. Therefore, we used creatinine clearance as a reasonably accurate estimate of GFR, although we recognize that creatinine clearance has limitations and does not exactly measure GFR.

To our knowledge, this is the first study assessing the performance of serum cystatin C as a marker of GFR in an adequately large cohort of renal transplant recipients. In our group of unselected patients, there was a wide variety in length of time from transplantation to study, immunosuppressive maintenance regimes, and biopsy results. Unlike most previous studies, the present one fulfills the methodological standards required for the evaluation of diagnostic marker [18], and our nonselected cohort covers the entire range of renal transplant function. This may permit some generalization of our data regarding the performance of cystatin C.

In summary, the results of this study suggest that serum cystatin C accurately reflects creatinine clearance over the entire range of transplant function. Serum cystatin C is as efficacious as serum creatinine to detect reduced creatinine clearance in renal transplant recipients. However, contrary to previous studies, cystatin C presents no advantage over creatinine in renal transplantation.

Acknowledgements We kindly thank Rita Haase and Kornelia Litznerski for excellent measurement of serum cystatin C, serum and urine creatinine concentrations, Martin Strnat for reviewing the manuscript, and Dade Behring Corp. for the generous provision of cystatin C kits.

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