Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C

Lorenz Risch, Alfred Blumberg and Andreas Huber

Department of Laboratory Medicine and Division of Nephrology, Kantonsspital Aarau, Switzerland

Abstract

Background. Assessment of renal function in patients with renal transplants is of great importance. Various studies have reported cystatin C as an easily and rapidly assessable marker that can be used for accurate information on renal function impairment. To date, no study is available to define the role of cystatin C in patients with renal transplants.

Methods. Thirty steady-state patients (50% male/50% female) with status post-kidney transplantation were studied. To assess renal function, cystatin C, creatinine clearance, serum creatinine, $\beta_2$-microglobulin ($\beta_2$M), and $[^{125}]$iodothalamic clearance were determined. Correlations and non-parametric ROC curves for accuracy, using a cut-off glomerular filtration rate (GFR) of 60 ml/min, were obtained for the different markers allowing for calculations of positive predictive values (PPV), positive likelihood ratios (PLR), specificity and sensitivity, respectively. Further, to evaluate the usefulness of these markers for monitoring, intraindividual coefficients of variation (CVs) for cystatin C and creatinine measurements were compared in 85 renal transplant patients. Measurements consisted of at least six pairs of results, which were obtained at different time points during routine follow-up.

Results. Cystatin C correlated best with GFR ($r = 0.83$), whereas serum creatinine ($r = 0.67$), creatinine clearance ($r = 0.57$) and $\beta_2$M ($r = 0.58$) all had lower correlation coefficients. The diagnostic accuracy of cystatin C was significantly better than serum creatinine ($P = 0.025$), but did not differ significantly from creatinine clearance ($P = 0.76$) and $\beta_2$M ($P = 0.43$). At a cut-off of 1.64 mg/l, cystatin C has a PPV of 93%, PLR of 6.4, specificity 89% and sensitivity 70%, respectively. For $\beta_2$M, PPV 83%, PLR 1.7, specificity 67% and sensitivity 75% was seen at a cut-off of 3.57 mg/l. Accordingly, at a cut-off of 125 μmol/l for serum creatinine, a PPV 76%, PLR 1.4, specificity 44% and sensitivity 80% was revealed. Finally, at a cut-off of 66 ml/min/1.73 m² for creatinine clearance, the follow-

Key words: cystatin C; glomerular filtration rate; intraindividual variability; kidney transplantation; renal function markers; ROC curve

Introduction

Assessment of renal function in clinical medicine is of great importance, especially in patients with renal transplants. For this purpose, serum creatinine levels currently are used. However, serum creatinine has significant disadvantages such as an inability to measure renal function impairments of 50% or less [1]. In addition, the commonly used method, according to Jaffe, and its modifications are plagued by a multitude of analytical interferences [1,2]. Thus, serum creatinine can only be used as a crude indicator of a significantly impaired renal function, <50% of normal [1]. Furthermore, rapid changes in glomerular filtration rate (GFR) are not detected [1]. In order to determine GFR decreases, creatinine clearance determinations have been used. However, according to Perrone et al. [1], creatinine clearance is not useful in determining the exact level of renal function. It is only of use in
determining whether renal function is nearly normal, moderately or severely reduced. In addition, due to secretion in the tubular system, creatinine clearance leads to a significant overestimation of GFR in patients with decreased GFR [3,4]. Furthermore, collection of an accurate 24-h urine is laborious and poses additional sources of errors. Taken together, although creatinine is a cheap and simple test for GFR assessment, it allows only crude estimation of renal function as it is hampered by many biological and technical problems.

To date, many accurate methods for determining GFR have been described, including inulin clearance and clearances of $^{125}$Iiothalamate, $^{51}$CrEDTA and iohexol. Unfortunately, these methods are extremely laborious and complicated and are thus not used for routine measurements of GFR. For these reasons, many attempts at finding a better marker have been undertaken, but not until recently, when cystatin C was described, has there been a valuable marker available. Cystatin C, an inhibitor of cystein proteinases, has the characteristics of an ideal marker to assess renal GFR [5]. Cystatin C is a low molecular weight basic protein (13.26 kDa) synthesized by all nucleated cells at a constant rate (housekeeping gene product) [5,6]. Cystatin C is a product of the cystatin gene superfamily of cystein proteinase inhibitors [7]. The endogenous production rate is constant, and is not affected by inflammatory processes, changes of body mass, nutrition, fever or gender [8]. Infants older than 1 year have stable cystatin C levels [9]. Only a slight increase in cystatin C is seen in ageing humans consistent with a decrease of GFR with age [8]. Thus, due to stable synthesis, lack of degradation and tubular secretion, cystatin C is only influenced by renal GFR, thus making it an ideal marker.

Rapid and fully automated, accurate methods for determination of serum cystatin C have been developed recently [10,11]. The analytical specificity and precision of serum cystatin C has been shown to be superior to serum creatinine determinations [10,11]. In addition, cystatin C has been reported to be a more sensitive marker of changes in GFR than serum creatinine [12]. Rapid and precise knowledge of renal GFR in patients with renal transplants is important. Deterioration of the transplant needs to be recognized early to avoid organ failure by adjusting the immunosuppressive medication. Furthermore, renally excreted medications need adjustment of dose as GFR changes.

So far, the relationship between the serum cystatin C, GFR and serum creatinine has only been established in healthy humans and in patients with renal problems caused by a variety of different diseases [5,12,13,14]. In order to assess whether serum cystatin C would be a reliable indicator for renal GFR in a well-defined group, we assessed this new marker in 30 stable patients with status post-renal transplantation. Cystatin C was assessed together with serum creatinine, creatinine clearance and $\beta_2$-microglobulin and compared with a gold standard, the $^{125}$Iiothalamate clearance [15]. Goerdt et al. have demonstrated that of many equations, two (e.g. Walser and Jelliffe) allow for the best approximation of GFR in renal transplant patients [16]. Thus, calculations using these equations were employed in our study. Finally, to demonstrate the characteristics as markers for monitoring, intraindividual variability of cystatin C and creatinine was compared in 85 renal transplant patients.

**Subjects and methods**

**Patients and samples**

Thirty patients under steady-state condition of post-renal transplant were included in the study. Steady-state was defined as lack of acute rejection periods during at least the past 6 months and stable cyclosporin A medication during the past 2 weeks. Gender was balanced with 15 male and 15 female patients, age distribution was $49 \pm 15.5$ years; time since transplantation was $6 \pm 4.5$ years, and $17\%$ of the patients were diabetic. Immunosuppression was with cyclosporin A and, in some patients, supplemented with prednisone. The determinations of serum creatinine, creatinine clearance, serum $\beta_2$-microglobulin and serum cystatin C were done during routine follow-up appointments. $^{125}$Iiothalamate clearance was determined after informed consent had been obtained. For the measurement of $^{125}$Iiothalamate clearance, the patients were hospitalized in the Nephrology Department of the Kantonsspital Aarau, where urine and plasma samples were taken by the experienced nephrology nursing staff. In order to calculate the intraindividual variability, serial measurements of cystatin C and creatinine in 85 renal transplant patients (47 male/38 female) were used. The results were all obtained in at least six pairs on different days during a 13-month follow-up. The study was approved by the ethics commission (IRB) of the Kantonsspital Aarau, according to the Helsinki Declaration of 1975.

**Laboratory methods**

Creatinine was measured on a Dimension (DuPont, Wilmington, DE) using a modified Jaffé method [17]. Interferences by bilirubin were avoided through addition of potassium ferrocyanide. Bichromatic measurements were done at 510 and 600 nm. Results were obtained 4 min after the start of the analysis. Creatinine was analysed in serum and 24-h urine to calculate the creatinine clearance related to $1.73 m^2$ body surface. In addition, estimation of the creatinine clearance was done according to the equations of Jelliffe and Cockroft-Gault, respectively, and estimate of GFR was obtained according to the equation of Walser.

Jelliffe equation [16]:

$$\text{Creatinine clearance (males)} = 98 - 16 \left(\frac{\text{age} - 20}{20}\right) + \text{plasma creatinine}.$$  

\[0.9 \text{ for females}\]

Cockroft-Gault equation [16]:

$$\text{Creatine clearance (males)} = (140 - \text{age}) \times \text{lean body weight} \div \text{serum creatinine} \times 72.$$  

\[0.85 \text{ for females}\]

Walser equation [16]:

$$\text{GFR} \times 3 \div \text{height}^2 = a + b \text{ (serum creatinine)}$$
Cystatin C was determined by using a particle-enhanced immunnoassay (PET) (Dako, Glostrup) using a Cobas Mira (Roche Diagnostics, Basel) [10]. Standard serum was used for this determination. Analysis was carried out at a wavelength of 340 nm. Results were obtained 7 min after starting the analysis. β2-Microglobulin was measured using an enzyme immunoassay (EIA) (Roche Diagnostics, Basel) employing a Cobas Core (Roche Diagnostics, Basel) [18]. Serum was also used for this analysis.

[125I]Iothalamate clearance was determined after allowing equilibration for 30 min after subcutaneous injection of 0.2 μCi/kg body weight by counting urine and plasma probes obtained within 2 min. Urine volume and concentrations of [125I]iothalamate in urine and plasma allowed the calculation of iohalamate clearance [15].

### Statistical methods

Reciprocal values of serum cystatin C, serum creatinine and β2-microglobulin as well as the creatinine clearance were compared with [125I]iothalamate clearances. The correlation was calculated according to Pearson. To quantitate the diagnostic value of the individual parameter, receiver operating curves (ROC) were obtained and analysed. To evaluate the use of cystatin C for severe and mild GFR, a cut-off was set arbitrarily at 60 ml/min. The area under the respective ROC curves was calculated according to the procedure of Hanley and McNeil [19]. With this, sensitivity, specificity, positive predictive value and positive likelihood ratio were calculated. The intraindividual coefficients of variation of cystatin C and creatinine and their ratios, respectively, were compared using the Mann–Whitney rank sum test. P-values <0.05 were considered significant. Data were obtained using an MS Excel (Microsoft Corporation, Seattle, WA) and a statistics program Analyze-It (Analyze-It Software Limited, Leeds, UK).

### Results

In order to evaluate the correlation of different markers with the GFR, comparison with the [125I]iothalamate clearance was carried out. As seen in Figures 1–4, reciprocals of the serum concentrations of cystatin C, β2-microglobulin and creatinine, respectively, were all found to increase with increasing GFR. The correlation coefficient (r) for 1/serum cystatin C was 0.83. As expected, correlation coefficients for creatinine clearance (r=0.57), 1/serum creatinine (r=0.67) and 1/serum β2-microglobulin (r=0.58) were lower than the value found for 1/cystatin C. Estimation of the creatinine clearance according to Jelliffe (r=0.25) and Cockcroft-Gault (r=0.10), respectively, and calculation of the GFR according to Walser (r=0.22) all revealed a weak correlation with [125I]iothalamate clearance.
Fig. 4. Relationship between creatinine clearance and GFR, as determined with \[^{125}\text{I}\text{iothalamate clearance.}\] Fifty five percent of creatinine clearance determinations overestimate GFR by >20%. Correlation coefficient is 0.57 (95% CI: 0.25–0.77). Linear regression line is: creatinine clearance = 3.75 + 1.23 \times \[^{125}\text{I}\text{iothalamate clearance.}\]

and therefore did not enhance the validity of serum creatinine values.

Further, to determine whether creatinine clearance provides accurate information on the GFR, deviation from \[^{125}\text{I}\text{iothalamate clearance was calculated as a percentage. The creatinine clearance differed <20\% in 10 patients, while a difference of >20\% was found in the remaining 19 patients. One patient dropped out due to technical problems. These data suggest that as many as two-thirds of patients with renal transplants have an inadequate determination of GFR, when assessed by creatinine clearance.

Furthermore, the diagnostic accuracy of the investigated parameters was assessed by calculation of the areas under the ROC curves, a commonly used assessment. As can be seen in Figure 5, 1/serum cystatin C had a significantly higher diagnostic accuracy than 1/serum creatinine using a cut-off of 60 ml/min GFR (P=0.024). Surprisingly, no significant difference of the areas under the ROC curves comparing 1/serum cystatin C with creatinine clearance (P=0.76) and 1/\beta_2\text{-microglobulin (P=0.517) was observed.}

To describe the characteristics of the analysed parameters, sensitivity, specificity, positive predictive value and positive likelihood ratio were calculated. The results are listed in Table 1 showing cystatin C as having similar properties in assessing GFR when compared with creatinine clearance.

Comparison of intraindividual coefficients of variation revealed significantly lower values for creatinine than cystatin C (P<0.001) (Figure 6). Calculating the ratios between coefficients of variation for creatinine and cystatin C shows most individuals to have a value <1. However, individuals with cystatin C concentrations >1.8 mg/l (n=43) have significantly higher ratios than individuals with concentrations <1.8 mg/l (n=42) (P=0.019). With increasing concentrations of cystatin C, the ratios tend towards a value of 1, expressing the equality of the coefficient of variation for cystatin C and creatinine at lower GFR.

Discussion

In patients with status post-renal transplantation, rapid assessment of the transplant function (GFR) is necessary. This allows for early recognition of rejection and accurate dosing of different simultaneously used drugs that show renal excretion or renal toxicity. A precise measurement of GFR can be done using clearances of inulin or radioactive markers. All these methods, however, are time consuming and cumbersome for both patients and staff. The use of endogenous markers is thus of great advantage due to speed and simplicity. To date, creatinine clearance has been felt to be the best method available. However, the method is plagued with pre-analytical compliance problems, analytical imprecision and biological variation of creatinine synthesis and elimination [1]. As seen in our study and reported by others, creatinine clearance overestimates the true clearance in many cases [1,4]. Use of serum creatinine for a rough estimate is plagued by interference through muscle mass changes, nutrition, physical activity and inflammatory processes [1]. Taking all these factors, serum creatinine and creatinine clearance allow a very crude estimation of renal function.

Our results demonstrate clearly that of all available markers, cystatin C has the best correlation with GFR. Like Keevil et al. [20] who suggested that cystatin C is superior to serum creatinine, we demonstrate with these data the usefulness of cystatin C as a test for detecting renal impairment. Cystatin C is superior to
It was surprising that creatinine clearance did be rea...GFR [8,18].

Additional, because of compliance problems, creatin- the management of renal transplant patients, because

To be of use for monitoring renal function, any parameter is expected to have a low intraindividual variation. Keevil et al. [20] have reported that cystatin C has a larger intraindividual variation than serum creatinine, as evaluated in 12 healthy volunteers. Our study is the first to confirm these findings for renal transplant patients. As cystatin C is a more sensitive marker of changes in GFR than creatinine [12], the broader variability of cystatin C could be attributable to this fact, reflecting small temporary changes of GFR more accurately than creatinine. Moreover, coefficients of variation of cystatin C and creatinine tend to equalize with increasing concentrations of cystatin C, suggesting that creatinine and cystatin C have the same variability. With a low GFR, both markers accurately reflect renal function, while in mild renal function impairment, creatinine is not sensitive enough to assess transient changes, thus incorrectly indicating a low variability.

Therefore, the pseudo-high variability of cystatin C in mildly reduced GFR is rather a good assessment of a biologically rapidly changing GFR. A changing cystatin C result in a range close to normal should be regarded as a warning sign of unstable renal function that could deteriorate rapidly. From this aspect, the use of cystatin C as a screening and monitoring test is strengthened further. However, these findings have to be reaffirmed further by studies comparing a gold standard method with the serum measurements of cystatin C and creatinine in mild GFR impairment patients.

Taken together, our results show that cystatin C is superior for renal assessment in a well-defined patient group. This is in accordance with previous studies indicating a superiority of cystatin C in less well-defined patient groups. From these studies, however, it cannot be assumed that cystatin C is of benefit in the management of renal transplant patients, because in the two previously investigated collectives the proportion of patients with renal transplants was only 11% [5,14]. Therefore, to date, it is unclear whether a possibly weak correlation of cystatin C and GFR in transplant patients was masked by the remaining patients with different nephropathies. Because rapid assessment of renal function with an accurate marker is of considerable importance in management of patients with renal transplants, our investigation in this patient group was thus justified.

**Table 1.** Diagnostic test characteristics of serum cystatin C, serum creatinine, serum β2-microglobulin and creatinine clearance in renal transplants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-off value</th>
<th>PPV (%)</th>
<th>PLR</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>1.64 mg/l</td>
<td>93</td>
<td>6.4</td>
<td>89</td>
<td>70</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>3.57 mg/l</td>
<td>83</td>
<td>1.7</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>Creatinine</td>
<td>125 μmol/l</td>
<td>76</td>
<td>1.4</td>
<td>44</td>
<td>80</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>66 ml/min</td>
<td>94</td>
<td>7.7</td>
<td>89</td>
<td>85</td>
</tr>
</tbody>
</table>

Sensitivity, specificity, positive predictive value (PPV) and positive likelihood ratio (PLR) are shown.

**Fig. 6.** Ratio of intraindividual coefficients of variation (CVs) for creatinine and cystatin C in 85 renal transplant patients. CVs for creatinine are significantly lower than for cystatin C (P<0.001). Individuals with a mean cystatin C level >1.8 mg/l (n=43) have significantly higher ratios than individuals with mean cystatin C <1.8 mg/l (n=42) (P=0.019). With higher cystatin C concentrations, ratios tend towards a value of 1, indicating an equal CV for cystatin C and creatinine.

serum creatinine, even when the equations of Cockroft-Gault, Walser and Jelliffe are used, which correct for age, weight, height and gender, respectively [16]. Compared with creatinine clearance and β2-microglobulin, no significant differences in accuracy were found. It was surprising that creatinine clearance did not differ significantly from cystatin C. However, due to the fact that our patients were in steady-state, it can be assumed that the study was biased against cystatin C. It is expected that during inflammatory processes or other additional conditions, especially during acute rejection or infections, both common problems in patients with renal transplants, cystatin C would continue to provide a precise assessment of GFR while creatinine clearance would vary dramatically. Additionally, because of compliance problems, creatin-ine clearance performed under out-patient conditions would be expected to be of lower diagnostic value than cystatin C. Further, the serum concentration of β2-microglobulin, another low molecular weight protein, is influenced by its production rate and the GFR [8,18]. Its production, however, is dramatically different in patients with lymphoproliferative syndromes, infections and autoimmune diseases [18]. In addition, immunosuppressive drugs will change the rates of β2-microglobulin production, making it an impractical candidate for a GFR marker in patients with renal transplants.

Serum cystatin C in patients with renal transplants

1995

![Diagram](image)
Cystatin C is clearly superior to serum creatinine, with a positive likelihood ratio of 6.4 and a positive predictive value of 93%, respectively. We conclude that the greater variability of cystatin C compared with creatinine might be due to the higher sensitivity in reflecting small temporary changes of GFR, especially in mildly impaired renal function. Cystatin C has similar characteristics as creatinine clearance, however, is not plagued by 24-h urine collection and its well-known problems with compliance. It remains to be seen whether cystatin C is superior even in patients that are not in steady-state. Taken together, serum cystatin C determinations allow for a rapid and accurate assessment of renal function (GFR) in patients with renal transplants.

References


Received for publication: 9.9.98
Accepted in revised form: 23.4.99