Serum cystatin C performs similarly to traditional markers of kidney function in the evaluation of donor kidney function prior to and following unilateral nephrectomy

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Abstract

**Background and objectives.** It is essential that candidates for kidney donation be carefully screened to ensure safety of donation and monitored following unilateral nephrectomy as previous experience has demonstrated loss of kidney function of up to 50% following donation. The low-molecular-weight protein cystatin C (cysC) has been introduced as an alternative to serum creatinine for estimation of glomerular filtration rate (GFR). In particular, serum cysC is sensitive to detect mild GFR reduction between 60 and 90 ml/min/1.73 m² that would make it a potentially effective screening and monitoring test in live kidney donors.

**Design, setting, participants and measurements.** We examined the utility of cysC as compared to other traditional measures of kidney function, including serum creatinine and 24-h urine for creatinine clearance, in the evaluation of kidney function in 51 consecutive live kidney donors both prior to and following unilateral nephrectomy.

**Results.** This is the largest experience reported in the living kidney donor population. We found that living kidney donors at our centre lost ∼30–35% of kidney function following unilateral nephrectomy and this remained stable >1 year. Furthermore, we observed that cysC correlated well with all other markers of kidney function and detected acute changes in kidney function immediately post-nephrectomy.

**Conclusions.** Overall, however, cysC did not confer any advantage with respect to preoperative assessment of kidney function or for monitoring following live kidney donation as compared to more traditional measures.

**Keywords:** creatinine; cystatin C; glomerular filtration rate; kidney function; living donor

Introduction

Renal transplantation is the treatment of choice for end-stage renal failure as it results in improved patient survival and quality of life as compared to dialysis [1,2]. A problem encountered in deceased donor transplantation however is the scarcity of resources (donor organs) and this has necessitated examining other possible organ sources, in particular living donor kidney donation. Living donors have not been shown to have an increased risk of hypertension, proteinuria or renal impairment up to 20 years after donation [3–5]. However, it is essential that candidates for kidney donation be carefully screened to ensure that no harm from donation is incurred. It is equally important to follow kidney function in donors long-term to ensure stability and ensure early and accurate detection of decreasing GFR so as to prevent progression of chronic kidney disease and to potentially improve outcome.

Conventionally, serial measurements of serum creatinine and urinary creatinine clearance are used to evaluate and monitor renal function in prospective donors. However, it is now well established that serum creatinine alone is a relatively poor marker of GFR as it is affected by age, sex and muscle mass. Attempts to improve the accuracy of GFR estimation using serum creatinine have resulted in the development of creatinine-based GFR estimating equations such as the calculated Cockcroft–Gault (CG) and the Modification of Diet in Renal Disease (MDRD) formulas [6,7]; however, these equations have been shown to be less accurate in patients with a GFR >60 ml/min/m². Urinary creatinine clearance, while commonly used and often considered the gold standard, is inconvenient for patients and is often inaccurately collected. The use of radioisotope scans for estimation of GFR is invasive, expensive and not generally used for routine preoperative evaluation of renal function in the workup of living kidney donors.

Newer, more effective and relatively less expensive alternatives for screening for renal impairment are needed, however, until recently few options existed. Cystatin C (CysC) is a nonglycated basic housekeeping protein (MW 13 359) of the cystatin superfamily of cysteine proteinase inhibitors.
Cystatin C is cleared by renal glomerular filtration followed by tubular reabsorption, which catalyzes and degrades it completely. There are several advantages in using Cystatin C as a marker for GFR. It has a constant rate of production by nucleated cells; its production rate is unaltered under inflammatory conditions, and its low molecular mass allows it to be freely filtered by the glomerulus. In the general population, Cystatin C has been shown to be more sensitive than serum creatinine (SCr) in detecting subtle impairment in GFR earlier (88 ml/min versus 75 ml/min; \( P < 0.001 \)) [8]. This should make it particularly effective in evaluating renal transplant recipients and potential donors of live donor kidneys for subtle changes in renal function prior to and following donation.

As a new marker of renal impairment, Cystatin C has recently been examined in many different patient populations including the elderly [9], normal children [10], adult type 2 diabetics [11], patients with multiple myeloma [12], cancer patients [13], patients with essential hypertension [14], renal transplant recipients [15–17], patients with glomerulonephritis/various renal diseases [8,18–20] and patients with hepatic cirrhosis [21]. In many of these studies and in particular in the renal transplant recipient population, Cystatin C proved equivalent to radioisotope (125-I iothalamate) determination of GFR and 24-h urine for creatinine clearance and was superior to SCr for monitoring impairment in renal function post-transplant. A meta-analysis of 24 studies examining the clinical utility of Cystatin C revealed that 15 studies concluded that Cystatin C is superior to SCr, whereas, 9 concluded that Cystatin C is equivalent but provides no advantage [22]. The summary ROC plot analysis of 20 studies that provide sensitivity and specificity data strongly suggested that Cystatin C will be superior to SCr for detecting impaired GFR.

In this study, we examined 70 subjects who were evaluated as potential live kidney donors between 1 July 2002 and 31 August 2004 to define the change in kidney function following unilateral nephrectomy and to examine the correlation between Cystatin C and the following tests at baseline (Tc-99 only available at baseline) and post-nephrectomy at Day 2, Month 1 and Year 1: (1) SCr; (2) 24-h urine creatinine clearance (CrCl); (3) calculated CG CrCl and (4) calculated MDRD GFR.

### Methods

Approval from the University of Alberta Hospital Health Research Ethics Board was obtained prior to study initiation. All potential living kidney donors identified through the University of Alberta Live Kidney Donor Workup Program were approached to participate in the study. Of the 70 subjects consenting to this study, 51 proceeded through the donation process, while the other 19 subjects were either rejected as potential donors for medical/psychological reasons (\( n = 8 \)), replaced by another donor (\( n = 2 \)) or the recipient did not proceed with live donor transplantation (\( n = 9 \)). The data of the latter 19 subjects were collected up until the point of termination of their workup and were only used for baseline comparison. While the results did not differ significantly when these 19 individuals were excluded, only data related to the 51 subjects that underwent donation are presented.

Each potential donor underwent a series of investigations as part of the routine evaluation process at baseline (in duplicate, 1 week apart) that included blood work (haemoglobin and serum creatinine), technetium-99 nuclear medicine GFR (Tc-99 GFR once only), 24-h urine collection and blood pressure measurement. For the purposes of this study, each participant also had Cystatin C drawn at baseline and postoperatively on Day 2, Month 1 and Year 1. All subjects were to be followed for 1 year post-nephrectomy.

The DAKO Cystatin C particle-enhanced turbidimetric assay on the Hitachi 917 was used to determine the Cystatin C level at the University of Alberta Hospital laboratory. Serum creatinine measurements were performed using provincie-wide standardized assays.

### Statistical analysis

SPSS 15.0 was used to perform statistical analysis. A \( P < 0.05 \) was considered for statistical significance. The Cystatin C, GFR by Cystatin C, SCr, MDRD GFR, CG and 24-h urine CrCl were either measured or calculated at the baseline and postoperative Day 2, Month 1 and Year 1. First, we compared these measurements between preoperative and postoperative Day 2 by the non-parametric Wilcoxon signed-rank test. Second, we focused on the postoperative changes over time; comparisons were performed by using Friedman ANOVA; post hoc tests were performed using the Wilcoxon signed-rank test and \( P \)-values were corrected using Bonferroni corrections for multiple pairwise tests. Pearson correlations were computed between 1/Cystatin C and Tc-99 GFR (baseline only), 1/SCr, 24-h urine CrCl, CG and MDRD GFR. Receiver operator characteristic (ROC) curves were constructed based on 24-h urine CrCl changes from baseline to Month 1 and from baseline to Year 1, using a cutoff of change in CrCl \( \geq 40 \) ml/min versus \(< 40 \) ml/min. Formulae for calculation of creatinine clearance were used as previously described for CG [6], MDRD [7] and Cystatin C-based calculation of GFR [28].

### Results

Of the 51 subjects evaluated in this study, 25\% (\( n = 13 \)) were male and the average age was 40.4 ± 11 years. Self-reported donor ethnicity was reported as Caucasian 80\% (\( n = 41 \)); Aboriginal 14\% (\( n = 7 \)); Asian 4\% (\( n = 2 \)) and Spanish 2\% (\( n = 1 \)). The average baseline haemoglobin was 141 ± 12 g/l. The average baseline blood pressure was systolic 121 ± 9 and diastolic 76 ± 6 mmHg. Missing data post-nephrectomy existed for two patients at Day 2, two patients at Month 1 and eight patients at Year 1. None of the donors had thyroid disease, were current smokers or were on corticosteroids.

There was a significant decrease in the renal function of living kidney donors as measured by all markers of renal function following unilateral nephrectomy (\( P < 0.01 \); Table 1). On average, renal function declined by...
Table 1. Comparisons of serum creatinine (SCr), 24-h urine creatinine clearance (CrCl), cystatin C, GFR by cystatin C, Cockcroft–Gault (CG) and MDRD measurements between preoperative and postoperative Day 2 (n = 49 with complete data)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Postoperative Day 2</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCr (µmol/l)</td>
<td>73 ± 15</td>
<td>106 ± 22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-h urine CrCl (ml/min)</td>
<td>120 ± 27</td>
<td>78 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C (mg/l)</td>
<td>0.79 ± 0.13</td>
<td>1.01 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR&lt;sup&gt;b&lt;/sup&gt; by cystatin C (ml/min)</td>
<td>129 ± 31</td>
<td>86 ± 23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CG&lt;sup&gt;c&lt;/sup&gt; (ml/min)</td>
<td>118 ± 29</td>
<td>81 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MDRD&lt;sup&gt;d&lt;/sup&gt; (ml/min)</td>
<td>92 ± 18</td>
<td>59 ± 12</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Wicoxon signed-rank test P-values.
<sup>b</sup>GFR = 86.49 × Cystatin C × 1.186 × 0.948 (if female) [28].
<sup>c</sup>CG = (140 – Age) × Weight (kg) / SCr × 1.230 (if male).
<sup>d</sup>MDRD = 186 × SCr−1.154 × Age−0.203 × 0.742 (if female).

Table 2. Comparisons of serum creatinine (SCr), 24-h urine creatinine clearance (CrCl), cystatin C, GFR by cystatin C, Cockcroft–Gault (CG) and MDRD measurements postoperative Day 2, Month 1 and Year 1 (n = 40 with complete data)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD at postoperative time</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCr (µmol/l)</td>
<td>Day 2 72 ± 22</td>
<td>103 ± 22</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Month 1 80 ± 21</td>
<td>83 ± 21</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td>Year 1 82 ± 21</td>
<td>84 ± 21</td>
<td>0.595</td>
</tr>
<tr>
<td>24-h urine CrCl (ml/min)</td>
<td>Day 2 106 ± 22</td>
<td>101 ± 22</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Month 1 103 ± 22</td>
<td>101 ± 22</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Year 1 101 ± 22</td>
<td>101 ± 22</td>
<td>0.595</td>
</tr>
<tr>
<td>Cystatin C (mg/l)</td>
<td>Day 2 1.01 ± 0.16</td>
<td>1.15 ± 0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Month 1 1.15 ± 0.21</td>
<td>1.10 ± 0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Year 1 1.10 ± 0.21</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>GFR&lt;sup&gt;b&lt;/sup&gt; by cystatin C (ml/min)</td>
<td>Day 2 86 ± 22</td>
<td>71 ± 22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Month 1 83 ± 21</td>
<td>84 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Year 1 83 ± 21</td>
<td>84 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CG&lt;sup&gt;c&lt;/sup&gt; (ml/min)</td>
<td>Day 2 80 ± 19</td>
<td>81 ± 19</td>
<td>0.495</td>
</tr>
<tr>
<td></td>
<td>Month 1 81 ± 19</td>
<td>82 ± 21</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>Year 1 81 ± 19</td>
<td>82 ± 21</td>
<td>1.000</td>
</tr>
<tr>
<td>MDRD&lt;sup&gt;d&lt;/sup&gt; (ml/min)</td>
<td>Day 2 60 ± 12</td>
<td>62 ± 15</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Month 1 62 ± 15</td>
<td>64 ± 15</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>Year 1 64 ± 15</td>
<td>64 ± 15</td>
<td>0.595</td>
</tr>
</tbody>
</table>

<sup>a</sup>Friedman ANOVA test P-values.
<sup>b</sup>Post hoc multiple pairwise comparisons test Bonferroni corrected P-values.
Hypothesis: Friedman ANOVA test employed to evaluate whether a marker exhibits change over postoperative timepoints and post hoc tests allow which pair of three timepoints are different.

GFR = 86.49 × Cystatin C × 1.186 × 0.948 (if female) [28].
GFR = 140 – Age × Weight (kg)/SCr × 1.230 (if male).
MDRD = 186 × SCr−1.154 × Age−0.203 × 0.742 (if female).

~33–43 ml/min for donors by Day 2 post-unilateral nephrectomy. Donors lost at least 20, 30, 40 and 50 ml/min kidney function at Month 1 in 70, 58, 38 and 33%, respectively, and at Year 1 in 67, 49, 36 and 26%, respectively. This decline persisted at 1 year post-nephrectomy, and in some cases minor increase in GFR was observed (Table 2).

The average values at baseline for the specific kidney function tests were (Table 1) SCr 73 ± 15 umol/l; 24-h urine CrCl 120 ± 27 ml/min; Tc-99 GFR 94 ± 14 ml/min; CG CysC 73 ± 15 ml/min and Cystatin C 0.79 ± 0.13 mg/l. There was a significant decrease in the renal function of living kidney donors as measured by all markers of renal function from baseline to postoperative Day 2 (P < 0.001; Table 1). However, CysC was the only marker of renal function to detect a further change in renal function between postoperative Day 2 and Month 1 and Day 2 and Year 1 post-unilateral nephrectomy (Table 2). Baseline preoperative values of CysC correlated with the other renal function tests with the weakest relationship being between CysC and CG GFR (Figure 1).

Sensitivity analysis was performed using ROC curves to examine the ability of CysC to detect changes in GFR compared to the gold standard 24-h urine CrCl (reference category). Similar curves were constructed to compare CG CrCl and MDRD GFR to urinary CrCl to detect a change in kidney function of 40 ml/min or greater by postoperative Month 1 and Year 1 following unilateral nephrectomy. As shown in Figure 2, CysC performed similarly and was not superior to the 24-h urine CrCl, CG CrCl and MDRD GFR at 1 Month and 1 year following donation. Of note, GFR estimates based on CysC were performed similarly (data not shown).

Discussion

It is essential that candidates for kidney donation are carefully screened to ensure safety of donation as well as monitoring of renal function following unilateral nephrectomy given previous reports of loss of kidney function of up to 30–50% [3–5,25–27]. New methods of detecting renal impairment earlier and more accurately are constantly being sought out, such as CysC. CysC has been recently evaluated as a screening test for impaired GFR in the living kidney donor population in two small studies. Herget-Rosenthal et al. prospectively evaluated whether CysC detected a rapid GFR decrease earlier and more accurately than SCr in 10 patients undergoing nephrectomy for living-related kidney transplantation [23]. The authors found that CysC detects rapid GFR decreases 1–2 days earlier than creatinine.
However, the authors defined a GFR decrease as a 50–100% increase of CysC or creatinine as compared to preoperative values, which raises questions about the quality of donors in the study, sensitivity of the test and generalizability of this population’s results to other groups. In addition, subjects were followed for 4 days and this limited follow-up provides no information of long-term outcomes of kidney function. Furthermore, John et al. examined 28 living kidney donors to compare the estimation of GFR based on CysC, SCr and serum B2 microglobulin drawn, on average, 7 days prior and 10 days following unilateral nephrectomy for kidney donation. The authors found that because of its large intra-individual variation, serial CysC estimation was very poor in detecting reduced renal function as compared to SCr or serum B2 microglobulin [24]. Both studies were limited by small sample size, and neither study had the long-term follow-up of renal function beyond 10 days that is important to evaluate the long-term status of renal function post-nephrectomy.

In this study, we examined 51 living kidney donors to define the change in function following unilateral nephrectomy and to compare CysC as a screening test for detecting change in kidney function with other traditional markers of renal function for up to 1 year following donation. This is
the largest cohort of living kidney donors evaluated to our knowledge.

We found that the average change in renal function following unilateral nephrectomy is 30–35%, which is similar to previous reports [25–27]. However, of interest, we did not observe early hyperfiltration with eventual loss of function that is likely an accurate observation in the ‘human model’; rather we observed an early loss of 30–35% that remained stable over the 1-year follow-up. Furthermore, we observed that CysC is more sensitive to acute changes in renal function immediately post-nephrectomy, it confers no advantage with respect to preoperative assessment of renal function or for long-term post-nephrectomy monitoring.

A potential limitation of this study is that urinary CrCl was used as our reference gold standard for estimation of GFR following nephrectomy. However, the strength of the pre-transplant correlation between Tc-99 GFR and CysC was not different from the other markers of renal function. Radioisotope scans may be more accurate in GFR estimation; however they are not routinely used, and therefore these results represent current clinical practice.

In summary, we observed a fixed loss of kidney function of 30–35% following living kidney donation with the 1-year follow-up. CysC is the only marker suggestive of meaningful postoperative changes after Day 2, whereas other kidney function markers remain unchanged. While changes up to 1 month after unilateral nephrectomy may exist, neither standard serum markers nor measured creatinine clearance indicates significant postoperative changes of kidney function for up to 1 year. We recommend a continued use of the traditional measures of kidney function in preoperative screening and a postoperative follow-up of living kidney donors while other new markers of kidney function can be developed and evaluated.

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