Cystatin C Levels in Functionally Anephric Patients Undergoing Dialysis: The Effect of Different Methods and Intensities

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Background and objectives: Cystatin C, a low molecular weight protein, is produced by nucleated cells, filtered by glomeruli, and degraded by tubules at a constant rate. Its serum concentration has been proposed as a marker of GFR. Its size should make it dialyzable. It is hypothesized that serum cystatin C levels are influenced by the method and intensity of dialysis received.

Design: This is a cross-sectional pilot study of cystatin C in functionally anephric dialysis patients. It was measured predialysis in 14 patients on conventional (3 to 5 h, 3 × wk) hemodialysis; eight on nocturnal hemodialysis (three to seven nights, 6 to 8 h); three on daily hemodialysis (6 d, 1½ to 2½ h); and 10 on automated peritoneal dialysis. All had urea kinetic studies and values for single pool Kt/V (Sp Kt/V), standard weekly Kt/V (Std Kt/V), and protein equivalent of nitrogen appearance (nPNA; g/kg/d). C reactive protein (CRP; mg/L) and thyroid stimulating hormone (TSH; mIU/L) were measured as factors known to influence cystatin C.

Results: There was no correlation between cystatin C and Sp Kt/V, but there was a significant inverse linear correlation with Std Kt/V and there were significant differences between treatment modalities in cystatin C levels and in Std Kt/V. The estimation of cystatin C was reliable and stable over 3 to 6 wk and its levels uninfluenced by nPNA, CRP, or TSH.

Conclusion: Serum cystatin C levels are influenced by the method and intensity of dialysis and may have a role in treatment adequacy monitoring.


Serum creatinine is a widely used yet crude marker of GFR (GFR). The limitations of serum creatinine and creatinine clearance for estimation of GFR are well known (1). Creatinine concentration is affected by several factors that are independent of GFR, such as age, race, muscle mass, gender, medication use, and catabolic state (2). Serum cystatin C, a cystine protease inhibitor, is a low molecular weight protein (13.2 KD) produced at a constant rate by all nucleated cells (3). In the kidney, it is freely filtered and catabolized in the proximal tubule without being secreted (3). Studies suggest that cystatin C is a better marker of GFR than serum creatinine because of its independence from age and gender (4). Prediction equations have been derived from pediatric and adult patients to give an estimate of GFR from the serum cystatin C (5,6). Surprisingly there are few studies of serum cystatin C levels in dialysis patients. Its size (13.2 kDaltons) should make it dialyzable and a marker for “middle molecule” toxin removal. We, therefore, conducted a pilot study of serum cystatin C levels in such patients. A recent study by Delaney and colleagues suggested that serum cystatin C reflected predominantly renal not dialytic clearance in chronic renal failure patients on peritoneal dialysis (7). For this reason, we studied functionally anephric patients. We hypothesized that serum cystatin C levels would be related to markers of dialysis adequacy such as the standard weekly Kt/V urea (Std Kt/V) (8). To test this hypothesis, we studied patients treated by a variety of dialytic modalities that provided a range of values for Std Kt/V. Significant differences in Std Kt/V exist between conventional three times per week hemodialysis and daily or nightly hemodialysis (9). Std Kt/V can also be used to compare different treatment modalities (peritoneal versus hemodialysis) as well as different frequencies and treatment times (8). We studied patients encompassing all these treatment modalities.

Materials and Methods

This was a cross-sectional pilot study in patients with ESRD receiving therapy by dialysis at the London Health Sciences Centre (Canada). The study was approved by The University of Western Ontario Ethics Review Board (#15348E), and all patients gave written informed consent. The patients selected were all known to be functionally anephric.
with residual urine volumes of 0 to 250 ml/d. They were enrolled from the following cohorts: those receiving conventional 3 × wk hemodialysis (CHD) with each treatment lasting 3 to 5 h; patients undergoing short hours daily hemodialysis (SHDHD) at home. These patients dialyze 5 to 7 d per wk each time for 2 to 3 h; those receiving home nocturnal hemodialysis 3 to 7 nights/wk with each treatment lasting for 6 to 8 h (NHD); and patients treated with automated peritoneal dialysis (APD) using a total dialysate volume of 10 to 15 L overnight plus a daytime fill of 2 L.

All patients were medically stable at the time of selection and had been on their specific form of therapy for at least 3 mo without alteration of dialysis prescription. As previous studies to help calculate an appropriate study sample size remain elusive, we aimed for approximately 10 patients in each treatment group. Fourteen out of the first 20 (of a total of 194 CHD patients) who were identified as being anephric volunteered. There were only seven patients doing SHD; three were anephric and volunteered. There were 17 patients doing NHD, 12 were anephric, of whom eight agreed to participate. Finally, of 150 APD patients, the first 20 patients known to be functionally anephric were contacted and 10 volunteered. This gave a total of 35 patients.

All hemodialysis patients were dialyzed by the same high flux polysulphone membrane dialyser (Optiflux 160 Fresenius, Toronto, Canada). Venous or, in the case of fistulas, arterialized blood samples for cystatin C were collected predialysis from all hemodialysis patients. In the case of CHD and thrice weekly NHD patients, they were taken midweek. With the other NHD patients, they were taken predialysis at any time. The NHD patients were trained to centrifuge and then store the bloods overnight at 4°C to be collected the following morning. With the APD patients, blood samples were taken at a clinic visit.

Cystatin C levels were measured by immune nephelometry using an N-latex cystatin C kit (Dade Behring, Mississauga, Canada) on a Behring BN ProSpec analyser (Dade Behring Marburg, Germany). The co-efficient of variation (CV) of the serum cystatin C measurement has been previously established at 3.1% at 1.06 mg/L, 3.5% at 2.04 mg/L, and 6.7% at 5.26 mg/L. Cystatin C levels were drawn in duplicate in most patients to enable measurement reliability assessment, and in the CHD patients, another sample was drawn 3 to 6 wk after the first to assess stability over time. This was not done with the SHDHD, NHD or APD groups to minimize inconvenience. The cystatin C was given as an absolute level in mg/L. As there have been reports of cystatin C elevation associated with inflammation and C reactive protein (CRP) rise and with thyroid dysfunction (11,12), at the time of cystatin C sampling, blood samples were also taken for CRP and thyroid stimulating hormone (TSH) levels. CRP was measured by immunonephelometry (Dade Behring BN Prospec, Mississauga, Canada) with CV of 4.02% at the level of 12.79 mg/L and 4.48% at 50.87 mg/L. TSH was measured by immunonephelometry (Dade Behring BN Prospec, Mississauga, Canada) with CV of 2.7% at the level of 4.02 mIU/L and 3.6% at 20.04 mIU/L. The nature of the interrelationships between variables of interest was assessed by means of a scatterplot was used to ensure that measurement stability was preserved at all magnitudes of cystatin C. In addition, a paired t test was used to demonstrate that the difference between time points was small and not statistically significant.

Differences between treatment modalities for the different variables studied were analyzed using parametric or nonparametric ANOVA testing of Variance. Gender differences were assessed by Pearson χ². Differences between treatment modalities for the different variables studied were analyzed using parametric or nonparametric ANOVA testing of Variance. Gender differences were assessed by Pearson χ². The nature of the interrelationships between variables of interest was assessed by means of bivariate correlations. Bivariate linear regression analysis was used to further specify these inter-relationships, with scatterplots used to graphically present the regression lines.

Results

The total number of patients included in this study was 35 (45.7% females), and their age median was 57.0 (range 32.0 to 88.0). Details of patient demographics by age, gender, and weight (postdialysis target weight in kilograms) are summarized as follows for each mode of treatment: CHD patient median age was 65 yr, number of males to females was 7:7, and target weight 85.9 Kg; SHDHD patient median age was 36.3 yr, number of males to females was 2:1, and target weight 65.3 Kg; NHD patient median age was 44.8 yr, number of males to females was 6:2, and target weight 85.9 Kg; APD patient median age was 58.6 yr, number of males to females was 5:5, and target weight 71.8 Kg. Table 1 summarizes the descriptive statistics of all variables under study in the whole group of patients.

The CHD group (mean age 65) is statistically older than both the nocturnal HD group (44.75 yr; P = 0.008, t test) and the DHD group (36.33 yr; P = 0.009, t test). The APD group mean age is not statistically higher than the NHD or DHD groups. There were no differences by weight.

Table 2 gives the results for cystatin C levels (mg/L), SpKt/V, Std Kt/V, C reactive protein (CRP) (mg/L), TSH...
There were significant differences between groups for all parameters (min \( P < 0.008 \)) save the CRP and TSH results. The nocturnal group had a statistically significantly lower mean cystatin C level than both the CHD (\( P < 0.004 \)) and the APD (\( P < 0.002 \)) groups. The nocturnal group had a statistically significantly higher mean \( \text{Std Kt/V} \) than the daily group (\( P < 0.001 \)), the CHD group (\( P < 0.001 \)), and the APD group (\( P < 0.001 \)). The daily group had a statistically significantly higher mean \( \text{Std Kt/V} \) than the APD group (\( P < 0.017 \)). The nocturnal group also had a statistically significantly higher \( \text{nPNA} \) than the CHD (\( P < 0.009 \)) and the APD groups (\( P < 0.024 \)).

Cystatin C had a moderately strong statistically significant negative correlation with \( \text{StdKt/V} \) (\( r = -0.49; P = 0.003 \)). These data are also shown in Figure 1. The regression equation would indicate that for every increase in cystatin C of 1 mg/L, the \( \text{StdKt/V} \) decreased by 0.703. It was found that \( \text{nPNA} \) does not correlate significantly with cystatin C (\( r = -0.260; P = 0.166 \)), \( \text{SpKt/V} \) (\( r = 0.087; P = 0.65 \)), or \( \text{StdKt/V} \) (\( r = 0.648; P = 0.341 \)).

CRP and TSH levels showed no intercorrelations with any other measured parameter.

On 24 occasions, duplicate samples were taken for cystatin C estimation. When these samples were taken, the tubes were arbitrarily labeled as sample A and sample B. The values referred to above are for sample A. The mean value for sample A was 6.04 \( \pm 1.01 \) mg/L and for sample B, 5.95 \( \pm 1.11 \) mg/L (NS, paired \( t \) test). The mean value for the difference between paired samples was 0.09 \( \pm 0.57 \) mg/L (NS). There was also a strongly significant linear correlation between the two cystatin C results (\( r = 0.951; P < 0.001 \)). On 18 occasions, it was possible to have a predialysis cystatin C measured 3 to 6 wk after the initial samples were taken. The late values were compared with the sample A initially taken. The linear correlation between this late sample and sample A was not statistically significant (\( r = 0.368; P = 0.142 \)). The difference between the measures, however, remains small and statistically insignificant (\( \chi = 0.132 \) mg/L, \( P = 0.267 \); 95% confidence interval \( \pm 0.15 \) mg/L). In the whole population, median time difference between the urea kinetic

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### Table 1. Descriptive statistics of all variables studied in the entire group of 35 patients

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Median</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Shapiro Wilk Test ( p )-value</th>
<th>Normal?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.00</td>
<td>42.00</td>
<td>68.00</td>
<td>32.00</td>
<td>88.00</td>
<td>0.1043</td>
<td>Yes</td>
</tr>
<tr>
<td>Weight</td>
<td>81.60</td>
<td>72.10</td>
<td>94.00</td>
<td>48.50</td>
<td>150.0</td>
<td>0.0438</td>
<td>No</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>6.190</td>
<td>5.680</td>
<td>6.650</td>
<td>3.510</td>
<td>8.570</td>
<td>0.8495</td>
<td>No</td>
</tr>
<tr>
<td>Single pool Kt/V (SpKt/V)</td>
<td>1.690</td>
<td>1.470</td>
<td>1.900</td>
<td>0.6400</td>
<td>3.580</td>
<td>0.0011</td>
<td>Yes</td>
</tr>
<tr>
<td>Standard weekly Kt/V (Std Kt/V)</td>
<td>2.314</td>
<td>1.990</td>
<td>4.393</td>
<td>1.330</td>
<td>7.035</td>
<td>&lt;0.0001</td>
<td>No</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.815</td>
<td>2.713</td>
<td>14.65</td>
<td>0.5800</td>
<td>26.50</td>
<td>0.0322</td>
<td>No</td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>2.395</td>
<td>1.698</td>
<td>3.950</td>
<td>0.7200</td>
<td>22.61</td>
<td>&lt;0.0001</td>
<td>No</td>
</tr>
<tr>
<td>nPNA (g/kg/d)</td>
<td>1.065</td>
<td>0.8875</td>
<td>1.285</td>
<td>0.6600</td>
<td>2.720</td>
<td>&lt;0.0001</td>
<td>No</td>
</tr>
</tbody>
</table>

### Table 2. Data values by treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Nocturnal and daily</th>
<th>CHD</th>
<th>APD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C mg/L</td>
<td>5.3 (4.5, 5.9)</td>
<td>6.5 (6.0, 6.7)</td>
<td>6.4 (6.0, 7.5)</td>
</tr>
<tr>
<td>SpKt/V</td>
<td>1.7 (1.2, 2.0)</td>
<td>1.6 (1.4, 1.8)</td>
<td>1.9 (1.8, 1.9)</td>
</tr>
<tr>
<td>Std Kt/V</td>
<td>4.7 (4.4, 5.4)</td>
<td>2.3 (2.2, 2.4)</td>
<td>1.9 (1.6, 1.9)</td>
</tr>
<tr>
<td>CRP mg/L</td>
<td>6.5 (3.1, 10.8)</td>
<td>13.5 (8.4, 16.0)</td>
<td>2.6 (0.9, 20.3)</td>
</tr>
<tr>
<td>TSH mIU/L</td>
<td>2.1 (1.5, 3.4)</td>
<td>2.4 (1.4, 3.7)</td>
<td>3.9 (2.1, 4.3)</td>
</tr>
<tr>
<td>nPNA g/kg/d</td>
<td>1.3 (1.1, 2.0)</td>
<td>1.0 (0.9, 1.2)</td>
<td>1.0 (0.9, 1.3)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Data summarized as median (25th percentile, 75th percentile).

\( ^{b} \) As there were only three patients undergoing daily hemodialysis, their data values are merged with those undergoing nocturnal hemodialysis.

\( ^{c} \) CHD, Conventional 3 × wk hemodialysis.

\( ^{d} \) APD, Automated peritoneal dialysis.

(mIU/L), and \( \text{nPNA} \) (gm/kg/d). These are given by patient groups. There were significant differences between groups for all parameters (min \( P = 0.008 \)) save the CRP and TSH results. The nocturnal group had a statistically significantly lower mean cystatin C level than both the CHD (\( P = 0.004 \)) and the APD (\( P = 0.002 \)) groups. The nocturnal group had a statistically significantly higher mean \( \text{Std Kt/V} \) than the daily group (\( P < 0.001 \)), the CHD group (\( P < 0.001 \)), and the APD group (\( P < 0.001 \)). The daily group had a statistically significantly higher mean \( \text{StdKt/V} \) than the APD group (\( P = 0.017 \)). The nocturnal group also had a statistically significantly higher \( \text{nPNA} \) than the CHD (\( P < 0.009 \)) and the APD groups (\( P < 0.024 \)).
Cystatin C concentrations reflected predominately the renal and not peritoneal clearance. Hoek et al. (17) found similarly that cystatin C gave a good estimate of residual GFR in cohorts of both HD and PD patients. Filler and colleagues have shown in both children and adults with chronic kidney disease that an estimated GFR (eGFR) can be derived from the following formula: eGFR = 10 exp (1.962 + [1.23 × log (1/cystatin C)] (6,18).

Therefore, a knowledge of the Std Kt/V and the cystatin C level in a dialyzed patient with residual renal function might theoretically allow a knowledge of both dialytic and residual renal clearance: the Std Kt/V providing an “expected” cystatin C; a lower value indicating a finite eGFR. Delaney and colleagues (7), in their study, also measured nPCR (nPNA). They found a weak but significant positive correlation between these two parameters and postulated a relationship between nutritional status or muscle mass with cystatin C levels, even though the latter are generally considered to be uninfluenced by body mass (19,20). Our study did not show any relationship between StdKt/V and nPNA, thus eliminating the latter as a possible confounder. There is evidence that cystatin C levels themselves are reflective of outcome. Menon and colleagues (21) showed that serum cystatin C level is associated with all cause and cardiovascular disease mortality in stage 3 or 4 chronic kidney disease patients. If serum cystatin C levels are also found to correlate with outcomes in the dialysis population, they may become attractive indicators of treatment adequacy regardless of the presence or absence of residual renal function. Of course, the target range of a satisfactory cystatin C level remains elusive. Of note, Delaney and colleagues (7) suggested a similar concept.

Other information from this study is of relevance. The serum cystatin C levels showed no correlation with CRP or TSH. Furthermore, there was no difference between patient groups in mean CRP or TSH levels. Previous reports have suggested that cystatin C levels may be elevated in hyperthyroidism and with markers of inflammation (11,12). Furthermore there is no association between CRP levels and nPNA. We may assume that the higher PNA levels in the nocturnal dialysis patients are due to better nutritional status than secondary to inflammation-induced catabolism.

There is a limitation to this study in that not all the urea kinetic measurements were made on the same day that the serum cystatin C level was taken. Our test-retest observations show in the immediate sense that there is an excellent correlation between the measured levels found in the duplicate samples taken initially. Three to 6 wk after the first samples were taken, a further set of serum cystatin C values were taken in some patients. When compared with the initial levels, the close linear correlation that the initial duplicate samples had was lost. On the other hand, the mean difference between the initial and late values was only 0.13 mg/L with 95% confidence limits of ± 0.15 mg/L; this would not alter the statistically significant differences in cystatin C levels or standard weekly Kt/V values between the groups, nor the linear correlation between those two sets of parameters. This suggests that cystatin C levels are fairly stable in the dialysis population and perhaps depend upon a certain consistency of dialysis dose over time rather than on the presence or absence of residual renal function.
than be influenced by a single treatment. The fact that they are not influenced by the SpKt/V supports this concept.

In conclusion, we have shown that cystatin C levels are different in patients undergoing treatment by different forms of dialysis. The lowest levels are found in patients undergoing NHF and highest in patients treated by APD. Furthermore, there is a significant inverse relationship between the serum cystatin C level and the dose of dialysis given as measured by the Std Kt/V (Figure 1). Cystatin C is of a molecular size that should be dialyzable. The inference is that the differences in levels are due to its removal by dialysis. An alternative but unlikely possibility is that the patients studied had by chance different unknown co-morbidities that specifically influenced serum cystatin C levels and, at the same time, their selection to a specific treatment group (e.g., NHF versus APD). Not matching the treatment groups by demographic and known co-morbidities is (potentially) another limitation of this pilot cross-sectional study. Further studies into serum cystatin C levels across dialysis and its appearance in dialysate are indicated, as are further studies in dialysis patients who have residual renal function. Further correlations with outcomes are also necessary. The potential exists that serum cystatin C may be an ideal marker for both the monitoring of delivered dialysis intensity and of the overall adequacy of uraemia management provided.

Disclosures
None.

References