GFR meets mTOR: value of different methods to measure and estimate GFR & (side) effects of mTOR inhibition in renal transplantation
Baas, M.C.

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Cystatin C in Critically Ill Patients Treated with Continuous Venovenous Hemofiltration

M.C. Baas¹,², C.S.C. Bouman¹, F.J. Hoek³, R.T. Krediet², M.J. Schultz¹,⁴

¹Department of Intensive Care Medicine, ²Division of Nephrology, Department of Medicine, ³Department of Clinical Chemistry and ⁴Laboratory of Experimental Intensive Care and Anesthesiology, Academic Medical Center, University of Amsterdam, Amsterdam.

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ABSTRACT

Background Assessment of residual renal function in critically ill patients with acute renal failure (ARF) treated with continuous venovenous hemofiltration (CVVH) is difficult. Cystatin C (CysC) is a low molecular weight protein (13.3 kD) removed from the body by glomerular filtration. Its serum concentration has been advocated for assessment of renal function in patients with kidney disease.

Objective To investigate whether the removal of CysC by CVVH is likely to influence its serum concentration.

Patients and Methods Concentrations of CysC were measured in three consecutive samples in 18 patients with oliguric ARF treated with CVVH (2 L/h). Samples were taken from the afferent and efferent blood lines and from the ultrafiltrate line.

Results Concentrations of CysC did not change during the studied time interval. The mean serum concentrations of CysC were 2.25 ± 0.45 mg/l in the afferent and 2.19 ± 0.56 mg/l in the efferent samples (NS); ultrafiltrate concentrations of CysC were 1.01 ± 0.45 mg/l. The sieving coefficient of CysC was 0.52 ± 0.20; clearance of CysC was 17.3 ± 6.6 ml/min; removed quantity of CysC averaged 2.13 mg/h.

Conclusion During CVVH (2 L/h) the removed quantity of CysC is less than 30% of its production and no rapid changes in its serum concentration are observed. Therefore CVVH (2 L/h) is unlikely to influence serum concentrations of CysC significantly which suggests that it can be used to monitor residual renal function during CVVH.
INTRODUCTION

Critically ill patients are at risk of acquiring acute renal failure (ARF). Its incidence is 15-20% of all intensive care admissions of which 4-6% require some form of renal replacement therapy. During renal replacement therapy adequate monitoring of renal function is severely hampered because plasma creatinine and urea are removed by the hemofilter. This problem could be overcome by the use of Cystatin C (CysC).

CysC has been advocated as a marker of renal function. It is a low molecular weight protein (13.3 kD), produced at a constant rate by nucleated cells and removed from the body by glomerular filtration. CysC production is not influenced by sex, age, bodyweight or muscle mass and several studies have shown that CysC is a more sensitive indicator of mild reductions of renal function than creatinine. Moreover CysC was not removed during intermittent hemodialysis or continuous venovenous hemodiafiltration. However, critically ill patients with ARF will often receive continuous venovenous hemofiltration (CVVH). In contrast to hemodialysis, removing substances by diffusion, clearance of solutes with hemofiltration is achieved by convection (ultrafiltration) and adsorption. With the use of conventional membranes convection is associated with higher removal of middle and high molecular weight molecules.

The aim of this study was to investigate whether the removal of CysC by CVVH is likely to influence its serum concentration. If not, serum concentrations of CysC can be used as a marker for GFR in patients treated with CVVH.

METHODS

Patients

The present study was conducted as part of a wider study on the effect of CVVH on antimicrobial dosing. The study was conducted in a 28 beds multidisciplinary closed format intensive care in a university hospital and was performed in accordance with the guidelines of the local ethics committee. Consecutively admitted critically ill patients who required CVVH for oliguric ARF of any cause, in whom antimicrobial therapy was started to treat a known or suspected infection were included.

Continuous venovenous hemofiltration

Hemofiltration was performed with computer controlled fully automated hemofiltration machines (Diapact, Braun, Melsungen, Germany). Vascular access was obtained by cannulation of the femoral, jugular or subclavian vein using the Seldinger technique and a double lumen catheter (GamCath, Gambro, Hechingen, Germany). A 1.9 m² cellulose triacetate hollow fiber membrane (in vitro cut-off 60 kD, ultrafiltration coefficient 37 ml/h/mmHg, sieving coefficient for β2-microglobulin 0.81) was used (CT-190G, Baxter Healthcare Corporation, IL, USA). The extracorporeal circuit was anti-coagulated with heparin (Heparin Leo, Leo Pharma, Ballerup, Denmark). In
case of severe contraindications for anticoagulation, hemofiltration was performed without anticoagulation. Blood flow rate was 150 ml/min and warmed substitution fluids (SH 19, B-Braun; SB 53-HEP, B-Braun) were administered in predilution mode at a flow rate of 2 L/h.

**Samples**

Samples were obtained from the afferent (pre-hemofilter) and efferent (post-hemofilter) line of the extracorporeal circuit and from the ultrafiltrate line. They were collected at three different time points, 1.5 to four hours apart, between two successive intravenous gifts of antibiotics. Serum and ultrafiltrate were stored at -80°C.

**Assay**

CysC was measured with the N Latex Cystatin C test kit, a particle-enhanced immunonephelometric method, on a BN ProSpec analyser (Dade Behring, Leusden, the Netherlands). Normal serum CysC values range from 0.50 to 0.96 mg/l. Urea and creatinine concentrations were measured by standard clinical chemical methods.

**Calculations**

The prefiler serum concentration was multiplied by the dilution factor to correct for the dilution effects of predilution hemofiltration.

\[
\text{Dilution factor} = \frac{Q_b}{Q_b + Q_{inf}}
\]

Where \(Q_b\) is the blood flow rate and \(Q_{inf}\) is the infusion rate of the substitution fluid.

The following equations were used to calculate the sieving coefficient (SC), CVVH clearance (\(Cl_{CVVH}\)) and total mass removed in ultrafiltrate (\(M_{uf}\)):

\[
SC = \frac{2 \times C_{uf}}{C_{aff} + C_{eff}}
\]

\[
Cl_{CVVH} \text{ (mL/min)} = SC \times Q_{uf}
\]

\[
M_{uf} \text{ (mg/h)} = C_{uf} \times Q_{uf}
\]

Where \(C_{uf}\) is the concentration in ultrafiltrate and \(C_{aff}\) and \(C_{eff}\) are the concentrations in the afferent and efferent blood line respectively.

**Statistics**

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows version 11 (SPSS, Chicago IL, USA). Results are presented as mean ± SD. To examine changes over time, we used linear mixed models. This analysis studies average changes in subjects, taking into account the association between variables for individual patients at separate time points. To compare afferent and efferent values, we used the paired T-test. Differences at the level of \(P < 0.05\) were considered to be statistically significant.
RESULTS

Eighteen patients were included (Table 1). Per patient three afferent, three efferent and three ultrafiltrate samples were available. In one patient only two afferent samples were available and in two patients only two efferent samples were present. Figure 1 shows the afferent CysC concentrations in the three consecutive samples. Changes over time were not statistically significant for CysC, creatinine and urea. Therefore the mean individual data were used for further analysis and are summarized in Table 2. Figure 2 shows the individual data for CysC. The SC for creatinine averaged 0.9 and that of urea 1.0. The mean SC for CysC was 0.47. When corrected for possible incomplete mixing by using the SC of urea, which should be 1.0 by definition, a value of 0.52 ± 0.20 was found. Likewise, the corrected CysC hemofilter clearance was 17.3 ± 6.6 ml/min (Figure 3).

Serum CysC concentration was 2.21 ± 0.48 mg/l in the 12 patients receiving corticosteroids and 2.34 ± 0.41 mg/l in the others (NS).

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics (n=18). Values are mean ± SD or number.</th>
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<tbody>
<tr>
<td>Male / Female</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td>Urine production (ml/24 hrs)</td>
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<tr>
<td>APACHE-II score</td>
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<tr>
<td>Admission type</td>
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<tr>
<td>medical</td>
</tr>
<tr>
<td>surgical</td>
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<tr>
<td>Corticoid therapy</td>
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</tbody>
</table>

Figure 1. CysC concentrations in three consecutive afferent samples (horizontal lines represent mean ± SD). No statistically significant differences were observed over the three collection periods (P = 0.334).
**Table 2.** Concentrations, sieving coefficient, hemofilter clearances and removed quantity of CysC, creatinine and urea. Mean values ± SD.

<table>
<thead>
<tr>
<th></th>
<th>CysC</th>
<th>creatinine</th>
<th>urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afferent concentration</td>
<td>2.25 ± 0.45 mg/L</td>
<td>166 ± 99 μmol/L</td>
<td>13.0 ± 5.6 mmol/L</td>
</tr>
<tr>
<td>Efferent concentration</td>
<td>2.19 ± 0.56 mg/L</td>
<td>154 ± 90 μmol/L</td>
<td>12.4 ± 5.3 mmol/L</td>
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<tr>
<td>Ultrafiltrate concentration</td>
<td>1.01 ± 0.45 mg/L</td>
<td>160 ± 124 μmol/L</td>
<td>13.5 ± 9.0 mmol/L</td>
</tr>
<tr>
<td>Sieving coefficient</td>
<td>0.47 ± 0.19</td>
<td>0.90 ± 0.45</td>
<td>1.00 ± 0.45</td>
</tr>
<tr>
<td>Clearance</td>
<td>15.8 ± 6.5 ml/min</td>
<td>30.1 ± 14.9 ml/min</td>
<td>33.3 ± 15.0 ml/min</td>
</tr>
<tr>
<td>Removed quantity</td>
<td>2.13 ± 0.95 mg/h</td>
<td>340 ± 263 μmol/h</td>
<td>28.5 ± 19.0 mmol/h</td>
</tr>
</tbody>
</table>

**Figure 2.** Mean individual CysC concentrations in the afferent and efferent samples in 18 patients.

**Figure 3.** Mean corrected clearance of CysC by CVVH. Clearance was calculated from the urea-corrected sieving coefficient (horizontal lines represent mean ± SD).

**DISCUSSION**

In the present study the SC for CysC is 0.52 and the CVVH clearance is 17 ml/min. Adsorptive removal is unlikely because there is no difference in CysC level between the afferent and efferent concentrations. The removed quantity averages 2.13 mg/h. Data from literature show that the generation rate of CysC is 7.44 ± 1.44 mg/h\textsuperscript{16}. Consequently CVVH CysC removal is less than 30% of its generation and unlikely to influence serum CysC levels in a clinical significant way. This is confirmed by the absence of rapid changes in the CysC concentrations in individual patients.
Our study is the first study evaluating the removal of CysC during CVVH in critically ill patients with ARF. Balik et al.\textsuperscript{13} studied the effects of continuous venovenous hemodiafiltration (CVVHDF) with polysulphone membranes in critically ill patients and concluded that CysC is not removed during CVVHDF to a significant extent. However, solute removal during hemodialysis is based on diffusion and not convection as in CVVH.

Our study has several limitations. The conclusion is based on the generation rate of CysC as reported in the literature in non critically ill patients\textsuperscript{16}. Several conditions may have an effect on CysC levels, in particular thyroid disease\textsuperscript{17,18} and the use of corticosteroids\textsuperscript{19-21}. It is possible that the generation rate of CysC is influenced by critical illness; however this would affect our conclusion only in case of a reduced generation rate. In our study the difference in CysC levels between the patients with and without corticosteroids is not statistically significant, however the number of patients per group is small. We studied one filter and one ultrafiltration rate in the predilution mode. Membrane material and pore size might affect the SC for CysC. Convective removal of CysC was reported earlier during in vitro hemofiltration\textsuperscript{22}. In that study the SC for CysC was somewhat higher than in our study, most likely because high-cut-off membranes were used (in vitro cut-off = 100 kD). Moreover, adsorptive removal might be more prominent with other filters, in particular the polyacrylonitrile filter\textsuperscript{23}. In our study, applying an ultrafiltration rate of 2 L/h, the removal of CysC is not likely to affect serum levels. However, during the application of higher ultrafiltration rates, larger quantities of CysC will be removed and this may affect its concentration.

In conclusion, during CVVH (2 L/h) CysC is removed from the circulation; however the removed quantity is less than 30% of its production. Therefore CVVH (2 L/h) is unlikely to influence serum concentrations of CysC which suggests that it can be used to monitor residual renal function during CVVH.

REFERENCES


