Improved Prediction of Decreased Creatinine Clearance by Serum Cystatin C: Use in Cancer Patients before and during Chemotherapy

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Background: Serum cystatin C, a cysteine protease inhibitor, has been suggested as a new marker of glomerular filtration rate (GFR). This study explored the possibility of replacing the creatinine clearance (CrCl) estimation of GFR with cystatin C in early detection of renal impairment in cancer patients on chemotherapy.

Methods: Serum creatinine and cystatin C concentrations as well as 24-h CrCl were determined simultaneously in 72 cancer patients. Among them, 60 were treated with combined chemotherapy with cisplatin (CDDP). Creatinine was determined enzymatically with a spectrophotometric method. Serum cystatin C was determined by a particle-enhanced turbidimetric immunoassay. Serum creatinine C was determined by a particle-enhanced turbidimetric immunoassay.

Results: Cystatin C and creatinine correlated significantly (P = 0.001) with CrCl. The correlation was significantly better for cystatin C than creatinine (r = 0.84 vs 0.74; P = 0.01). Stepwise regression analysis identified no differences for the correlations between cystatin C and CrCl in patients with or without metastases (r = 0.82 and 0.84, respectively) as well as before treatment and before the fourth cycle of chemotherapy (r = 0.70 and 0.75, respectively). A cystatin C cutoff concentration of 1.33 mg/L had 87% sensitivity and 100% specificity for detecting CrCl < 78 mL/min. ROC analysis indicated that cystatin C was superior to serum creatinine for predicting CrCl < 78 mL/min (P < 0.04).

Conclusions: Serum cystatin C is superior to serum creatinine for detection of decreased CrCl and potentially for the estimation of GFR in cancer patients independent of the presence of metastases or chemotherapy.

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During treatment with chemotherapeutic agents such as cisplatin (CDDP),4 oncologists must monitor kidney function because alteration in renal function may lead to impaired metabolism and accumulation of chemotherapeutic agents and their metabolites (1). Because of proximal and, probably, distal tubular cell necrosis, the nephrotoxicity of CDDP is dose-related and cumulative; it depends on the extent of diuresis and preexisting alterations in renal function (2). Therefore, early recognition of significant renal injury is needed for safe and effective use of this agent.

The glomerular filtration rate (GFR) provides the best overall estimate of renal function. The urinary clearance of exogenous substances, such as 51Cr-EDTA and inulin, is accepted as the gold standard for the estimation of GFR. However, because of the cost and inconvenience, serum creatinine and creatinine clearance (CrCl) are the most widely used measures of renal function (3,4).

Serum creatinine is of limited value in early detection of renal insufficiency because it is well established that its concentration does not change significantly until CrCl is < 70 mL·min⁻¹·1.73 m² or inulin clearance is < 50 mL·min⁻¹·1.73 m²⁻² (4).

CrCl overestimates true GFR because creatinine not only is filtered by the glomeruli but also is secreted by the tubules. The contribution of tubular secretion to the total CrCl varies widely over time in patients and is increased in those with glomerular disorders (5). The measurement of CrCl involves a timed collection of urine, measurement of its volume, and determination of creatinine concentration in the urine and sera. Timed urine collections, however, are inconvenient for patients and are susceptible to errors (3,4). The usual 24-h CrCl may correlate less well with GFR than the estimates based on serum creatinine (3,6–8).

Cystatin C is a nonglycosylated 13-kDa basic protein of...
the cystatin superfamily of cysteine proteinase inhibitors with widespread distribution in biological fluids. It consists of 120 amino acids and is produced at a constant rate by all nucleated body cells (9, 10). Cystatin C is freely filtered in the glomeruli and, like other low-molecular mass proteins, almost completely reabsorbed and catabolized in the proximal renal tubular cells (11). Its serum concentration is determined mainly by GFR, and its renal plasma clearance is virtually identical to that of $^{51}$Cr-EDTA (12, 13). In addition, this protein is not significantly influenced by inflammation, and unlike creatinine, it is not affected by muscle mass and does not face the same problems with analytical interference as creatinine (14).

Several studies have indicated that cystatin C is a more sensitive indicator of decreased GFR than serum creatinine and could be an excellent replacement for exogenous markers and for creatinine in the estimation of GFR (13–17).

In the present study, we measured serum concentrations of creatinine and cystatin C and CrCl in patients with and without metastases, before and during chemotherapy. We also investigated the potential of cystatin C as a screening indicator for decreased CrCl in cancer patients on combined chemotherapy with CDDP.

**Patients and Methods**

We studied 72 patients (23 men and 49 women; mean age, 57 years; range, 27–77 years) with malignant melanoma, gastric cancer, and ovarian cancer (Table 1). Thirty-five patients had metastatic disease, and 37 patients were without evidence of disease at the time of treatment. All except 12 patients with impaired renal function were treated by combined chemotherapy with CDDP as adjuvant or palliative. The mean total dose of CDDP per cycle was 60 mg/m$^2$. Before treatment, all patients were without clinical evidence of severe systemic diseases, and they received no diuretics or drugs (e.g., cimetidine) known to interfere with creatinine secretion. Their body weight was 46–113 kg (mean, 73 kg), and their body surface was 1.5–2.1 m$^2$ (mean, 1.7 m$^2$).

**STUDY DESIGN**

Serum creatinine, cystatin C, and CrCl were determined simultaneously in all patients. In the patients with chemotherapy, they were measured before treatment and before the fourth cycle; in the patients without chemotherapy, they were determined 1 month after the first measurement. Altogether, 144 measurements were performed. The 24-h urine collection on an inpatient basis was carefully controlled by trained technicians. The patients were instructed to begin the collection of urine in the morning, to discard their first voided urine, and then to collect all of their urine for the next 24 h. In the morning of the second day, when urine collection was finished, blood samples for serum creatinine and cystatin C were taken before breakfast. CrCl was calculated using that morning creatinine.

**LABORATORY METHODS**

A Hitachi 911 automated analyzer was used to determine serum cystatin C and serum and urine creatinine concentrations. The serum and urine creatinine concentrations were determined enzymatically by a spectrophotometric method (Boehringer Mannheim). CrCl was calculated from urine flow, from the ratio between the serum and urine creatinine concentrations, and was standardized for the body surface area [CrCl = urine creatinine concentration ($\mu$mol/L) × urine flow (mL/min) × 1.73 m$^2$/serum creatinine concentration ($\mu$mol/L) × body surface area (m$^2$)].

The serum cystatin C was determined by immunohas-say using latex particle-enhanced immunoturbidimetry with a commercially available Dako cystatin C PET Kit (Dako A/S) (14). The reference values used in Slovenia for the serum concentrations of creatinine and CrCl in healthy individuals, independent of sex and age, are as follows: serum creatinine, 44–97 $\mu$mol/L; CrCl, 78–120 mL/min.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using Statistica for Windows software, Ver. 4.3, from StatSoft. In the evaluation of results, Pearson correlation coefficients were cal-

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**Table 1. Patient characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of patients</th>
<th>Measured values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Male/female</td>
<td>23/49</td>
<td></td>
</tr>
<tr>
<td>Median age, years</td>
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<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Metastases</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy with CDDP</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>CrCl, mL/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥78</td>
<td>25</td>
<td>97</td>
</tr>
<tr>
<td>&lt;78</td>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>Impaired renal function (CrCl ≤30 mL/min)</td>
<td>5/7</td>
<td></td>
</tr>
<tr>
<td>With/without metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, $\mu$mol/L</td>
<td>72</td>
<td>97</td>
</tr>
<tr>
<td>Before chemotherapy</td>
<td>60</td>
<td>94.2</td>
</tr>
<tr>
<td>Before the fourth cycle</td>
<td>60</td>
<td>90.8</td>
</tr>
<tr>
<td>With metastases</td>
<td>35</td>
<td>116.6</td>
</tr>
<tr>
<td>Without metastases</td>
<td>37</td>
<td>131</td>
</tr>
<tr>
<td>Serum cystatin C, mg/L</td>
<td>72</td>
<td>1.49</td>
</tr>
<tr>
<td>Before chemotherapy</td>
<td>60</td>
<td>1.31</td>
</tr>
<tr>
<td>Before the fourth cycle</td>
<td>60</td>
<td>1.26</td>
</tr>
<tr>
<td>With metastases</td>
<td>35</td>
<td>1.46</td>
</tr>
<tr>
<td>Without metastases</td>
<td>37</td>
<td>1.62</td>
</tr>
</tbody>
</table>
culated, and the significance of the difference between the correlation coefficients was estimated according to z-transformation (18). Because the serum concentrations of creatinine and cystatin C are inversely related to their clearances, the reciprocals of cystatin C and creatinine were calculated for the comparison with CrCl.

To assess the diagnostic accuracy of the serum concentrations of cystatin C and creatinine in predicting reduced CrCl, ROC plots (19) were performed, using MedCalc Software 4.3 for Windows (MedCalc). The area under the curve (AUC) and 95% confidence interval were calculated for each plot and compared by t-test.

**Results**

**CORRELATION BETWEEN CREATININE, CYSTATIN C, AND CrCl**

Among 72 patients, 12 had received no chemotherapy because their CrCl was <30 mL/min. As shown in Table 1, there were no significant differences in the serum creatinine and cystatin C concentrations between the group of 35 patients with clinical evidence of metastases, the group of 37 patients without clinical evidence of metastases, and the group of 60 patients before treatment and before the fourth cycle of chemotherapy.

The relationship between the serum concentrations of cystatin C or creatinine and GFR, as measured by CrCl, was analyzed according to the expectation of the ideal case where the serum concentration of cystatin C and creatinine should be inversely proportional to GFR. By plotting reciprocal values of serum cystatin C (y) or serum creatinine (y) vs CrCl (x), linear regression showed a significant relationship (P <0.001) between CrCl and both serum cystatin C and serum creatinine. The correlation was significantly better for cystatin C than creatinine (r = 0.84 vs 0.74; P = 0.01; Fig. 1).

**CORRELATION BETWEEN CYSTATIN C AND CrCl IN THE PRESENCE OR ABSENCE OF METASTASES AND DURING CHEMOTHERAPY**

The mean CrCl in the group of 35 patients with metastases and in the group of 37 patients without evidence of disease was 69 and 64 mL/min, respectively. CrCl did not differ significantly between the two groups of patients. High correlations (r = 0.82 and 0.84, respectively; P for difference = 0.7) between serum cystatin C and CrCl were found in both group of patients.

Among 60 patients with chemotherapy, CrCl was not significantly different before treatment and before the fourth cycle of chemotherapy. The mean CrCl before chemotherapy and before the fourth cycle was 72 and 70 mL/min, respectively. The correlation between cystatin C and CrCl before treatment (r = 0.70; P <0.01) was similar to and statistically indistinguishable from the correlation before the fourth cycle (r = 0.75; P <0.01; P = 0.6 for difference).

**SENSITIVITY AND SPECIFICITY OF SERUM CYSTATIN C AND CREATININE FOR PREDICTION OF GFR**

ROC plots for cystatin C and creatinine are presented in Fig. 2. The ROC analysis demonstrated a significant difference between the diagnostic efficiency of cystatin C and that of creatinine for the detection of CrCl <78 mL·min⁻¹·1.73 m⁻². The AUC for the cystatin C curve was significantly (P = 0.04) greater than that for the creatinine curve. The AUCs for the cystatin C and creatinine ROC plots were 0.967 and 0.761, respectively.

The cutoff at which the sum of sensitivity and specificity for CrCl <78 mL·min⁻¹·1.73 m⁻² was 1.33 mg/L for cystatin C (specificity, 100%; sensitivity, 87%) and 101 μmol/L for creatinine (sensitivity, 61%; specificity, 98%).
invasion and metastasis (22). Thus, the extent of malignancy may be thought to influence the cystatin C concentration. We have recently reported that the serum concentration of cystatin C was significantly higher in patients with advanced melanoma and colorectal cancer than in patients with primary melanoma and healthy controls. The creatinine concentration did not differ significantly between advanced cancer patients and healthy controls, but GFR was not estimated by CrCl in both groups of patients (23). Decreased protein intake or wasting of muscle mass in cancer patients may keep serum creatinine from increasing despite a decline in renal function related to age. In the present study, we did not find significant differences in the serum concentrations of cystatin C between the patients with and without metastatic disease. Our results confirm report of Khyse-Anderson et al. (14) that cystatin C is not influenced by any variable other than GFR. Our earlier results in a different group of patients may reflect alterations in renal function and not alterations directly related to malignant progression. Serum cystatin C concentrations started to increase to above the reference interval when CrCl was <94.4 mL/min, whereas serum creatinine remained within its reference interval (24). We also confirmed that combined chemotherapy with CDDP and cyclophosphamide; CDDP, ve-pesid, and doxorubicin; or CDDP, vinblastine, and recombinant interferon-α2a did not influence serum cystatin C concentration. The serum cystatin C concentration as well as the correlation between cystatin C and CrCl did not differ significantly before and after three cycles of chemotherapy.

The structure of the cystatin C gene and its promoter is of the “housekeeping type”, which is compatible with stable production of cystatin C (10). It is mostly present in extracellular fluid, and cell damage caused by chemotherapy is not likely to influence its serum concentration.

Nephotoxic cytostatic agents mainly damage tubular cells; therefore, they may interfere with the tubular absorption and metabolism of cystatin C. The urinary excretion of cystatin C in patients with tubular disorders is increased by up to three orders of magnitude (12). Because cystatin C, low-molecular mass protein, is filtered freely by glomeruli, and usually is almost completely reabsorbed and catabolized in proximal renal tubular cells (11), its serum concentration is dependent only on GFR.

The cutoff cystatin C concentration of 1.33 mg/L seems to be a good estimate of CrCl <78 mL/min. These results were similar to those obtained by others for an adult and a pediatric population (13, 17, 25, 26).

To prevent acute and chronic renal injury by CDDP, despite careful hydration, the dose of CDDP must be modified according to GFR estimated by CrCl. In general, the dose of CDDP in CrCl <60 mL/min must be reduced by one-half (1, 2). Our data show that in patients with normal or reduced renal function, the serum concentration of cystatin C was more informative than that of creatinine in the prediction of abnormal GFR. The high

Discussion
This study confirms the good correlation ($r = 0.84$) between the reciprocal of cystatin C concentration and CrCl in adults. Similar correlation coefficients of 0.71–0.87 were reported in studies where cystatin C was correlated to $51$Cr-EDTA or inulin clearance (9, 13–15). As in other studies, the correlation with the reciprocal of creatinine was significantly lower. The reported ratio of CrCl to GFR estimated by inulin clearance increases with decreasing GFR to ~1.7 at a GFR of ~20 mL/min (8, 20). When GFR is moderately impaired or normal, however, and urine collection errors were reduced, CrCl was nearly equal to GFR, with a ratio of CrCl to GFR of ~1.15 (6, 20, 21). In our study, the majority of patients had mild to moderate decreases in renal function, all patients were hospitalized, and urine collections were carefully monitored by trained technicians.

In patients with normal or moderately reduced renal function, the serum concentration of cystatin C seems to be more informative than that of creatinine (15). The serum creatinine concentration is influenced by non-renal factors (4, 8), and the day-to-day coefficient of variation in creatinine excretion may be as high as 70% when 24-h urine collection errors are not eliminated (7, 8). Cystatin C appears relatively unaffected by extrarenal factors such as sex, age, body mass index, dietary intake, and inflammatory conditions (14, 16).

In malignancy, an imbalance between cysteine proteases and their inhibitors, associated with metastatic tumor cell phenotype, is thought to facilitate tumor cell
sensitivity and specificity of cystatin C as well as its independence of other factors appear to make cystatin C equivalent to CrCl for early renal failure detection. Patients on nephrotoxic chemotherapy with serum concentration of cystatin C < 1.34 mg/L do not appear to need CrCl measurements. Moreover, because of many errors in outpatients measurement of CrCl, the use of cystatin C may be the more appropriate method for reduced GFR estimation than CrCl.

In conclusion, cystatin C may replace CrCl as a screening test before chemotherapy and perhaps may be used in dose modification of CDDP in patients with reduced GFR.

References