Original Article

Serum cystatin C as a marker of the renal function in patients with spinal cord injury

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Objective: To investigate the relationship between serum cystatin C, serum creatinine, and ⁵¹Cr-EDTA-clearance in patients with spinal cord injury.

Setting: The Spinal Cord Unit, Viborg-Kjellerup County Hospital.

Methods: Twenty-four men and seven women aged 20.3 to 68.0 years with motor complete spinal cord injury (ASIA A or B) were included. Serum cystatin C was measured by an automated particle-enhanced nephelometric immunoassay (Dade Behring), serum creatinine by an enzymatic method (Vitros 950), and ⁵¹Cr-EDTA-clearance by a multiple plasma sample method.

Results: A linear relationship was found between ⁵¹Cr-EDTA-clearance and the reciprocal values of cystatin C and creatinine. The correlation coefficient between ⁵¹Cr-EDTA-clearance and 1/cystatin C was 0.72 compared to the correlation coefficient between ⁵¹Cr-EDTA-clearance and 1/creatinine being 0.26. Comparison of the area under the curves in the non-parametric receiver operating characteristics (ROC) plots for serum cystatin C (area under the curve (AUC)=0.912; SE=0.065), and serum creatinine (AUC=0.507; SE=0.115) revealed significant differences (*P*-values=0.0005).

Conclusion: In patients with spinal cord injury serum cystatin C is a better marker of the renal function compared to serum creatinine.

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Keywords: creatinine; cystatin C; cysteine proteinase inhibitors; glomerular filtration rate; kidney function; spinal cord injury

Introduction

The measurement of serum creatinine and creatinine clearance is used as indirect markers of glomerular filtration rate (GFR). However, the concentration of serum creatinine is not an ideal parameter of GFR and glomerular filtration is only one of the parameters determining its serum concentration.¹ Renal handling and metabolism, food intake, and methodological interferences may influence the concentration of serum creatinine.^{2,3}

In patients with paraplegia and tetraplegia determination and monitoring of kidney function is of great importance and GFR is considered to be the best marker of renal function. Creatinine correlates with muscle mass¹ but in patients with spinal cord injury

(SCI) muscle mass is decreasing. Creatinine clearance determinations involve 24 h urine collection and may prove the accuracy but it is often difficult and time consuming for patients to perform.⁴ In patients with SCI urine collection is difficult and often inaccurate and creatinine clearance is encumbered with some uncertainties.

Cystatin C is a non-glycosylated low molecular weight protein ($M_r=13\,359$).⁵ It is produced by all nucleated cells at a constant rate,⁶ freely filtered in the glomeruli and reabsorbed and catabolized in the proximal tubular cells.⁷ The characteristics of cystatin C indicates that its serum concentration is mainly determined by GFR making cystatin C an endogenous parameter of GFR.^{7–13} Cystatin C is independent of gender, age (>1 year) and muscle mass.^{11,14–16}

The aim of this study was to investigate the relationship between serum cystatin C, serum creati-

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nine, and ^{51}Cr -EDTA-clearance in patients with spinal cord injury.

Materials and methods

Patients

Thirty-one patients with SCI classified ASIA A or B (24 men and seven women) aged between 20.3 and 68.0 years (40.4 ± 12.5 years; mean $\pm$ SD) were included. Patients with heart failure, kidney disease, malignancies and incomplete SCI (ASIA C-E) were excluded.

Sixteen patients had motor complete paraplegia. Twelve of the 16 patients had traumatic injury of the thoracic spine, one traumatic injury of the lumbar spine, one congenital myelomeningocele, one an epidural abscess (cervical five to thoracic one), and one patient had a transverse myelitis. Fourteen patients had traumatic complete tetraplegia, and one patient had an epidural abscess leading to tetraplegia.

Six patients did not receive any medical treatment. Of the remaining 25 patients four were treated with trimetoprim, and one patient was treated with steroids in low doses. None of the patients were treated with salicylates, phenacetamide, or cyclosporine. Several patients were treated with laxatives, muscle relaxants, and low molecular heparin. In the patients treated with trimetoprim, no differences of serum cystatin C and creatinine were found compared to patients without trimetoprim treatment.

The study protocol was approved by the local committee of ethics according to the Declaration of Helsinki and all the patients gave their written informed consent prior to the start of the study.

Procedure

During a period of $1\frac{1}{2}$ years, patients arrived at the Spinal Cord Unit, Viborg-Kjellerup County Hospital. Blood samples were drawn to analyze serum cystatin C, creatinine, urea nitrogen and albumin. A 24 h urine specimen was collected for determination of creatinine clearance. ^{51}Cr -EDTA-clearance was measured. Height and weight were registered.

Analytical methods

Blood samples were drawn from antecubital vein using Venoject VT-050UX tubes (Terumo-Europe, Leuven, Belgium). The blood samples were centrifuged at 1500 g for 15 min. Serum was isolated and analyzed on the same day, or within 2 months after storage at -80°C .

Serum cystatin C was analyzed using the Dade Behring N Latex Cystatin C assay on the Dade Behring Nephelometer II (Dade Behring Diagnostics, Marburg, Germany).¹⁷ The method was a fully automated particle-enhanced nephelometric immunoassay (PENIA). The assay time was 6 min for cystatin C compared to 5 min for creatinine and the

sample volume was 40 μL serum or plasma. The cystatin C assay could be performed along with 70 other protein assays (C-reactive-protein, immunoglobulins etc) on the Dade Behring Nephelometer II. The cost of a cystatin C test was approximately £2 compared to £0.2 for a creatinine test.

Serum and urine creatinine (enzymatic method), albumin, and urea nitrogen were analyzed on the Vitros 950 clinical chemistry system (Ortho-Clinical Diagnostics, Rochester, NY, USA).

GFR was measured by a single injection technique using ^{51}Cr chromium-ethylene-diamine-tetra-acetate complex (^{51}Cr -EDTA) by a multiple plasma sample method.¹⁸

Estimated GFR was calculated using the equation developed from the Modification of Diet in Renal Disease (MDRD) study.¹⁹

Creatinine-clearance and ^{51}Cr -EDTA-clearance was adjusted to a body surface area of 1.73 m².

Statistical analysis

Statistical analysis was performed using Student Edition of statistic version 2.0 for Windows 98 (Analytical Software, Tallahassee, FL, USA). Data are expressed as mean values $\pm$ SD. Correlation analysis was performed by calculation of Pearson's correlation coefficient (non-parametric). A *P*-value < 0.05 was considered significant. Receiver operating characteristics (ROC) plots were performed as described by Zweig *et al*²⁰ using GraphROCTM for Windows version 2.0 (developed by Veli Kairisto, Turku, Finland and Allan Poola, Tallin, Estonia).

Results

Patients' characteristics are listed in Table 1. The values are given as means $\pm$ SD. Serum cystatin C and serum creatinine showed an inverse relationship to ^{51}Cr -EDTA-clearance values (Figure 1a,b).

Serum cystatin C and serum creatinine and their relationship with ^{51}Cr -EDTA-clearance were linearized by plotting their reciprocal values (Figure 2a,b). There was a linear relationship between ^{51}Cr -EDTA-clearance and 1/serum cystatin C ($r = 0.72$; *P*-value < 0.0001), and between ^{51}Cr -EDTA-clearance and 1/serum creatinine ($r = 0.26$; *P*-value = 0.16).

Creatinine clearance was measured in 23 patients and showed decreasing values with decreasing ^{51}Cr -EDTA-clearance values (Figure 2c). There was a linear relationship between ^{51}Cr -EDTA-clearance and creatinine clearance ($r = 0.59$; *P*-value = 0.003).

Non-parametric ROC plots for serum cystatin C and serum creatinine are shown in Figure 3. Comparison of the areas under the curves (AUC) for serum cystatin C (AUC = 0.912; SE = 0.065) and serum creatinine (AUC = 0.507; SE = 0.115) revealed significant difference (*P*-value = 0.0005).

In 23 patients creatinine clearance was determined. AUC for serum cystatin C, serum creatinine, creati-

Table 1 Patients' characteristics. All data are expressed as mean values $\pm$ SD

	Men	Women	All
<i>n</i>	24	7	31
Age (year)	38.9 $\pm$ 11.3	45.4 $\pm$ 16.0	40.4 $\pm$ 12.5
Body mass index (BMI) (kg/m ²)	22.8 $\pm$ 3.52	23.5 $\pm$ 5.9	22.9 $\pm$ 4.1
Serum cystatin C (mg/L)	0.97 $\pm$ 0.35	0.95 $\pm$ 0.26	0.96 $\pm$ 0.33
Serum creatinine (μ mol/L)	69 $\pm$ 12	48 $\pm$ 9	65 $\pm$ 14
Creatinine clearance (mL/min/1.73 m ²) ^a	84.8 $\pm$ 21.3	62.6 $\pm$ 21.2	80.0 $\pm$ 22.8
Serum urea nitrogen (mmol/L)	4.2 $\pm$ 2.4	4.3 $\pm$ 1.5	4.2 $\pm$ 2.2
Serum albumin (g/L)	38.3 $\pm$ 4.7	33.9 $\pm$ 5.2	37.3 $\pm$ 5.1
⁵¹ Cr-EDTA-clearance (mL/min/1.73 m ²)	86.0 $\pm$ 20.5	91.3 $\pm$ 30.0	87.2 $\pm$ 22.5
Estimated GFR (mL/min) ^b	105.9 $\pm$ 22.0	109.7 $\pm$ 33.4	106.7 $\pm$ 24.6

^aTwenty-four hours creatinine clearance was only measured in 23 patients (18 men and five women). ^bThe estimated GFR has been calculated using the equation from the MDRD study¹⁹

Figure 1 Relationship between ⁵¹Cr-EDTA-clearance and (a) serum cystatin C and (b) serum creatinine in 31 patients. GFR $\geq$ 80 mL/min/1.73 m² was considered as a normal kidney function (vertical lines). (a) The upper limit of the reference interval for serum cystatin C was 1.02 mg L⁻¹ (horizontal). (b) The upper limit of reference interval for serum creatinine was 111 μ mol L⁻¹ for males (horizontal solid line) and 95 μ mol L⁻¹ for females (horizontal dotted line). Open circles = females, solid circles = males

nine clearance and estimated GFR values are listed in Table 2. Also, in this subgroup of patients significant difference was demonstrated between serum cystatin C and serum creatinine (P -value = 0.01). No significant difference was found between serum cystatin C and creatinine clearance or estimated GFR.

Discussion

The findings in the present study suggest that serum cystatin C is a better marker of the renal function than serum creatinine and creatinine clearance in adult patients with SCI.

Patients with spinal cord injury are at increased risk of developing renal insufficiency from several factors, eg high detrusor leak-point pressure, vesicoureteral reflux, recurrent ascending infection and renal calculi. The patients need to have their renal function examined on a regular basis.^{21,22} As a routine ⁵¹Cr-EDTA-clearance, creatinine clearance and serum creatinine is used for this purpose. ⁵¹Cr-EDTA-clearance is laboratory-intensive and time consuming.²³ The measured 24 h creatinine clearance is widely used but may be subject to error because of the inherent difficulty in collecting a 24 h urine specimen and laboratory error involved in measuring serum and urinary creatinine concentrations. Serum creatinine depends on the creatinine production. In patients with SCI serum creatinine is often significantly decreased as a result of the diffuse muscle atrophy which commonly accompanies muscle denervation.²⁴ In patients with SCI normal serum creatinine values could conceal a clinically significant reduction in GFR. Therefore it would be of value to have a simple, safe test to measure renal function.

The present study, including 31 patients with spinal cord injury, indicates that, serum cystatin C is a reliable parameter of GFR. In agreement with previous studies dealing with patients having different kidney diseases²⁵ serum cystatin C and creatinine increased with decreasing GFR values.^{23,25,26} Serum cystatin C correlated significantly better to GFR than

Figure 2 Relationship between ^{51}Cr -EDTA-clearance and (a) 1/serum cystatin C, (b) 1/serum creatinine, and (c) creatinine-clearance

serum creatinine and estimated GFR. ROC plots allow simultaneous comparison of the diagnostic specificity and sensitivity of serum cystatin C and creatinine. It can be used to evaluate the capability of serum levels of cystatin C and creatinine to correctly classify subjects into groups with normal or reduced GFR. A

Figure 3 Non-parametric ROC plots for serum cystatin C (solid line) AUC=0.912 and serum creatinine (dotted line) AUC=0.507

Table 2 Area under the curve (AUC) and standard error (SE) calculated using ROC plots

	n	AUC	SE
Serum cystatin C (mg/L)	23	0.893	0.086
Serum creatinine ($\mu\text{mol/L}$)	23	0.508	0.131
Creatinine-clearance (mL/min/1.73 m^2)	23	0.802	0.086
Estimated GFR (mL/min)	23	0.770	0.102

comparison of the area under the curves of the ROC plots for serum cystatin C and creatinine (P -values=0.0005) reveals significant difference, indicating that the diagnostic accuracy of serum cystatin C was superior to that of serum creatinine in identifying patients with reduced GFR.

Serum cystatin C levels can be determined by fully automated commercially available immunoassays which are rapid and precise.²⁷ No major interference with haemoglobin, bilirubin, triglycerides, and rheumatoid factors have been demonstrated using the assays.^{17,25} Reference intervals are established and the same reference interval can probably be used in children older than 1 year and in adults independent of gender.^{16,28} Furthermore, cystatin C is independent of muscle mass in contrast to creatinine. To our knowledge, no drugs have been reported to influence the serum levels of cystatin C in clinical practice.

Previous studies dealing with serum cystatin C as a parameter of GFR have suggested serum cystatin C to be as good a marker as serum creatinine^{11,26,28} or even superior to serum creatinine.^{8,10} Further studies demonstrated that in patients with normal to moderate

impaired kidney function serum cystatin C seemed to be superior to creatinine as a parameter of GFR.²⁸

In conclusion, the results demonstrate that serum cystatin C may be a better marker of the GFR than serum creatinine and creatinine clearance in patients with SCI. Serum cystatin C has the advantage that its concentration is independent of gender, muscle mass and age (>1 year) making cystatin C a promising parameter of the renal function in patients with SCI.

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