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Abstract *Purpose:* To evaluate
whether cystatin C in serum (sCyC)
and urine (uCyC) can predict early
acute kidney injury (AKI) in a mixed
heterogeneous intensive care unit
(ICU), and also whether these bio-
markers can predict the need for renal
replacement therapy (RRT). *Meth-
ods:* Multicenter prospective
observational cohort study in patients
$\geq$18 years old and with expected ICU
stay $\geq$72 h. The RIFLE class for AKI
was calculated daily, while sCyC and
uCyC were determined on days 0, 1,
and alternate days until ICU dis-
charge. Test characteristics were
calculated to assess the diagnostic
performance of CyC. *Results:* One
hundred fifty-one patients were stud-
ied, and three groups were defined:
group 0 ($N = 60$), non-AKI; group 1
($N = 35$), AKI after admission; and
group 2 ($N = 56$), AKI at admission.
We compared the two days prior to
developing AKI from group 1 with
the first two study days from group 0.
On Day –2, median sCyC was sig-
nificantly higher (0.93 versus
0.80 mg/L, $P = 0.01$), but not on
Day –1 (0.98 versus 0.86 mg/L,
$P = 0.08$). The diagnostic perfor-
mance for sCyC was fair on Day –2
[area under the curve (AUC) 0.72]
and poor on Day –1 (AUC 0.62).
Urinary CyC had no diagnostic value
on either of the two days prior to AKI
(AUC <0.50). RRT was started in 14
patients with AKI; sCyC and uCyC
determined on Day 0 were poor pre-
dictors for the need for RRT (AUC
$\leq$0.66). *Conclusions:* In this study,
sCyC and uCyC were poor biomark-
ers for prediction of AKI and the need
for RRT.

Keywords Urine cystatin C ·
Serum cystatin C ·
Acute kidney injury ·
Renal replacement therapy ·
Predictive biomarkers ·
Intensive care unit

Introduction

Acute kidney injury (AKI) is a common complication of critical illness and carries high mortality despite significant advances in medical care [1, 2]. This apparent lack of improvement may result from the use of more aggressive medical and surgical interventions in an ever-ageing population [3]. On the other hand, potentially effective therapeutic interventions for AKI may currently fail because they are applied late in the course of injury after an obvious increase of serum creatinine (sCr) is observed [4]. Due to the delayed rise in sCr following injury, recent efforts have focused on identification of an early and reliable biomarker of kidney injury [5, 6].

Cystatin C (CyC) is a 13-kDa, nonglycosylated basic protein, produced at a constant rate by all nucleated cells. It is freely filtered by glomeruli and catabolized in tubules. In high-risk patients, serum CyC (sCyC) detected AKI 1–2 days earlier than sCr [7]. Moreover, although CyC is normally not detected in urine, it has been found in urine of patients with tubular disease, suggesting that it is a tubular marker [8–13]. One drawback of use of CyC at present is a lack of recognition of its potential value for use in the general critical care setting, in which the population is heterogeneous and AKI etiology and timing are often unclear.

In the present study in a heterogeneous intensive care unit (ICU) population we collected serial samples of sCyC and uCyC, and determined the first day of AKI based on the RIFLE classification system [14]. We hypothesized that these markers predict AKI 1–2 days earlier than the RIFLE criteria in patients developing AKI after entry, and that these biomarkers predict the need for renal replacement therapy (RRT) on the first day of AKI. The preliminary findings of this study were presented in abstract form [15].

Concise methods

Patients

The protocol was approved by the institutional review board of all participating institutions, and written informed consent was obtained from all patients or their authorized representatives. The study was a prospective observational cohort study in adult patients with expected duration of mechanical ventilation of at least 48 h, and/or expected length of ICU stay of at least 72 h. Patients were enrolled within 48 h of ICU admission. Detailed methods (e.g., exclusion criteria) are described in Electronic Supplementary Material (ESM) file 1.

Demographic data, admission diagnosis, Acute Physiology and Chronic Health Evaluation (APACHE) II, and Simplified Acute Physiology Score (SAPS) II scores were documented upon ICU admittance [16, 17]. Routine laboratory data were measured daily.

Definition of acute kidney injury

The patients were scored daily for AKI using the creatinine and urine output criteria of the RIFLE classification system for AKI [14]. To define the baseline renal function we compared the premorbid sCr within 1 year prior to ICU admission with the sCr at ICU admission. The lower of these two values served as baseline renal function. If the premorbid sCr was unavailable, it was estimated by solving the modification of diet in renal disease (MDRD) equation as recommended by the Acute Dialysis Quality Initiative (ADQI) working group [18].

The premorbid sCr was not estimated in patients with history of kidney insufficiency. In these patients, admission sCr served as baseline when the premorbid sCr was unknown.

The first day of AKI was termed Day 0, while the two days prior to this day were termed Day −1 and Day −2, respectively.

Sampling and measurement of cystatin C

Blood and urine sampling for CyC measurements were performed on inclusion, day 1 and alternate days until the start of RRT, or ICU discharge. Samples were centrifuged at $1,500 \times g$ for 10 min at 4°C. The supernatants were stored at –80°C until assayed batchwise. Cystatin C was measured with an N Latex Cystatin C test kit, a particle-enhanced immunonephelometric method, on a BN ProSpec analyzer (Dade Behring, Leusden, The Netherlands). Urea and creatinine levels were measured by standard clinical chemical methods. We normalized urinary excretion of CyC for millimoles of urinary creatinine (uCyC$_{corr}$) to compensate for differences in urine flow rate [19].

Statistical analysis

The primary endpoint was the first RIFLE event (risk, injury or failure). The secondary endpoint was initiation of RRT. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS, Chicago IL, USA) for Windows. Continuous variables are expressed as mean ± standard deviation (SD) or median with interquartile range. Cystatin C levels below the detection limit of 0.05 mg/L were considered to be 0.025 mg/L. Categorical variables are expressed as counts and percentages. Normally distributed variables were compared using one-way analysis of variance with Bonferroni's correction for multiple comparisons. For significant findings, post hoc t-test was applied. Kruskall–Wallis one-way analysis of variance was used to compare non-normally distributed variables. Chi-square testing was used to test frequencies between groups. Linear

mixed models were used to compare CyC levels among RIFLE stages. All testing was two-tailed, and $P < 0.05$ was considered statistically significant. Test characteristics were calculated to assess the diagnostic performance of CyC.

Results

Patients

We enrolled 170 patients from April 2006 to November 2007 and excluded 19 patients during the analysis because of incomplete sampling, leaving 151 patients for analysis. One hundred thirty-three patients (88%) were included on the first day of ICU admission, and 18 patients (12%) were included the next day of admission. We defined three groups: group 0 ($N = 60$), never developed AKI, serving as controls; group 1 ($N = 35$), developed AKI after admission; and group 2 ($N = 56$), presented with AKI at admission. AKI developed after a median of 2 [1–2] days (range 1–7 days) in group 1: 17 patients (49%) were classified for AKI by the creatinine criteria, 14 patients (38%) by the urine criteria, and in 3 patients (9%) the creatinine and urine scores were identical. In group 2, 28 patients (50%) had AKI at admission by the creatinine criteria, 13 patients (23%) by the urine criteria, and in 17 patients the creatinine and urine scores were identical. Table 1 compares the baseline clinical data of the three

groups, while renal and outcome data are shown in Table 2. The maximum RIFLE class was higher in group 2 compared with group 1. In group 1 the maximum RIFLE class was achieved by the creatinine criteria in 21 patients (60%), by the urine criteria in 8 patients (23%), and in 6 patients (17%) the creatinine and urine AKI classification were identical. In group 2 the maximum RIFLE class was achieved by the creatinine criteria in 24 patients (43%), by the urine criteria in 13 patients (23%), and in 17 (30%) patients the creatinine and urine AKI classification were identical.

Serum and urinary cystatin C levels

Cystatin C was measured in 582 blood samples (3.8 ± 3.2 samples/patient) and 569 urine samples (3.8 ± 3.1 samples/patient). Cystatin C level was below the detection limit in 171 (30%) urine samples. Serum CyC levels increased significantly with increasing RIFLE class (Table 3). Urinary CyC levels also increased with increasing RIFLE class; however, the difference between RIFLE risk and RIFLE injury was not statistically significant.

Figure 1 compares the longitudinal trend of sCr, sCyC, and uCyC starting from 2 days prior to AKI (group 1) with the non-AKI trend (group 0). On Day –2, median sCyC was significantly higher (0.93 versus 0.80 mg/L, $P = 0.01$), but not on Day –1 (0.98 versus 0.86 mg/L, $P = 0.08$). Serum CyC levels, however, did

Table 1 Baseline characteristics grouped according to AKI status

	Group 0 ($N = 60$)	Group 1 ($N = 35$)	Group 2 ($N = 56$)	P
Age (years)	59.2 ± 16.1	68.4 ± 15.4	74.6 ± 9.3	<0.001
Male (%)	39 (65)	24 (69)	34 (62)	0.81
BMI (kg/m^2)	24.6 ± 4.3	26.5 ± 3.8	29.6 ± 12.8	0.11
APACHE II	18.5 ± 9.9	19.6 ± 8.3	24.2 ± 12.9	0.016
SAPS II	37.2 ± 13.3	44.1 ± 14.2	47.6 ± 16.4	0.01
Comorbidities (%)				
Hypertension	15 (26)	14 (40)	24 (44)	0.12
Diabetes	7 (12)	5 (14)	8 (15)	0.92
CKD	2 (3)	1 (3)	6 (11)	0.17
TSH (mU/L)	0.40 (0.18–0.87)	0.42 (0.25–1.0)	0.41 (0.21–1.20)	0.83
FT4 (pmol/L)	13.2 ± 3.5	12.8 ± 3.3	13.6 ± 3.6	0.67
Admission category (%)				0.68
Medical	29 (48.3)	17 (48.6)	23 (41.8)	
Surgical	31 (51.7)	18 (51.4)	33 (59)	
Primary diagnosis (%)				0.21
Cardiovascular failure	2 (3.3)	4 (11.4)	4 (7.1)	
Cerebrovascular event	2 (3.3)	0	0	
Hemorrhagic shock	7 (11.7)	2 (5.7)	6 (10.7)	
Multiple trauma	4 (6.7)	2 (5.7)	1 (1.8)	
Elective major surgery	1 (1.7)	1 (2.9)	5 (8.9)	
Respiratory failure	23 (38.3)	11 (31.4)	14 (25)	
Septic shock	20 (33.3)	13 (37.1)	26 (46.6)	

AKI acute kidney injury, *group 0* no AKI, *group 1* developed AKI after admission, *group 2* AKI at admission. *BMI* body mass index, *APACHE* Acute Physiology and Chronic Health Evaluation, *SAPS* Simplified Acute Physiology Score, *CKD* chronic kidney disease, *TSH* thyroid-stimulating hormone

Table 2 Renal characteristics and outcome grouped according to AKI status

	Group 0 (N = 60)	Group 1 (N = 35)	Group 2 (N = 56)	P
Premorbid sCr (μmol/L)				
Actual[a]	92 ± 30 (N = 37)	91 ± 25 (N = 28)	83 ± 23 (N = 37)	0.31
Estimated[b]	88 ± 12	86 ± 12	83 ± 11	0.05
Study inclusion				
sCr (μmol/L)	72 (61–94)	87 (76–87)	139 (97–211)	<0.001
sCyC (mg/L)	0.80 (0.65–1.04)	0.91 (0.81–1.17)	1.14 (0.88–1.94)	<0.001
uCyC (mg/L)	0.16 (0.025–1.47)	0.18 (0.07–0.68)	0.59 (0.10–3.47)	0.46
uCyC$_{corr}$ (g/molCr)	0.03 (0.01–0.30)	0.04 (0.01–0.13)	0.13 (0.01–0.67)	0.47
Maximum RIFLE class (%)				<0.001
Risk	–	27 (77)	22 (39.3)	
Injury	–	5 (14.3)	14 (25)	
Failure	–	3 (8.6)	20 (35.8)	
CVVH (%)	1 (1.7)	4 (11.4)	10 (17.6)	0.01
ICU treatment (days)	5 (3–8)	8 (5–18)	6 (3–9)	0.002
ICU mortality (%)	0	4 (11.4)	11 (19.6)	0.002
Hospital mortality (%)	4 (6.7)	6 (17.1)	18 (32.1)	0.002

AKI acute kidney injury, *group 0* never AKI, *group 1* developed AKI after admission, *group 2* AKI at admission, *sCr* serum creatinine, *sCyC* serum cystatin C, *uCyC* urine cystatin C, *uCyC$_{corr}$* urine cystatin C normalized for urinary creatinine, *CVVH* continuous venovenous hemofiltration, *ICU* intensive care unit

[a] Baseline serum creatinine based on premorbid serum creatinine within 1 year prior to ICU admission
[b] Baseline serum creatinine based on solving the modification of diet in renal disease (MDRD) equation [18]

Table 3 Cystatin C per RIFLE class

	None	Risk	Injury	Failure	P
Serum samples					
Number	399	109	35	31	
CyC (mg/L)	1.15 (1.05–1.25)	1.34 (1.22–1.45)	1.75 (1.59–1.92)	2.31 (2.09–2.52)	<0.001
Urine samples					
Number	391	104	40	34	
CyC (mg/L)	1.02 (0.54–1.51)	2.36 (1.52–3.19)	2.77 (1.43–4.12)	5.87 (4.24–7.51)	<0.001
CyC$_{corr}$ (g/mol Cr)	0.43 (0.28–0.59)	0.44 (0.21–0.68)	0.34 (−0.04–0.72)	0.79 (0.32–1.24)	0.46

Values are mean and 95% confidence interval. The P value represents the difference among groups by linear mixed models
sCyC serum cystatin C, *uCyC* urine cystatin C, *uCyC$_{corr}$* uCyC normalized for urinary creatinine
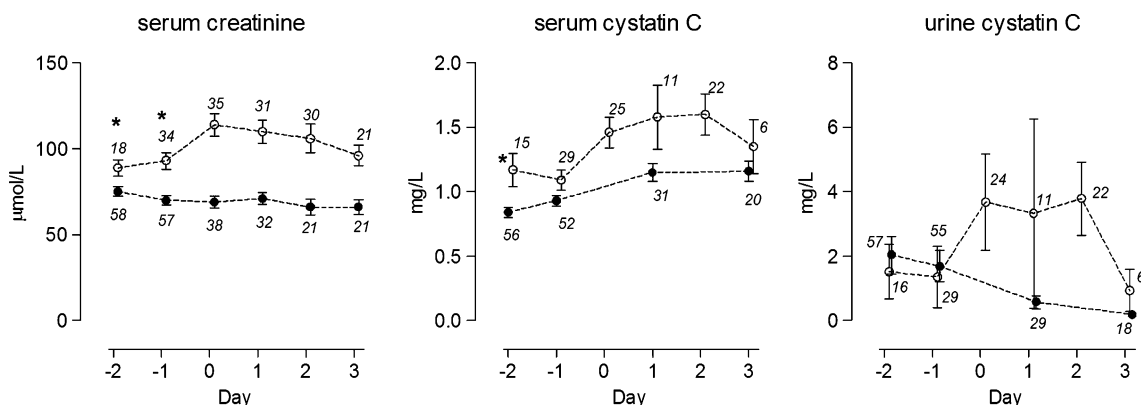

Fig. 1 Time course of serum creatinine, serum cystatin C, and urine cystatin C. Time courses are from 2 days prior to acute kidney injury (AKI) for patients developing AKI after entry (*open circles*), and from entry for the non-AKI group (*closed circles*). Values are mean and standard error of the means. The number of patients investigated is shown in *italics* at each time point. * $P < 0.05$ compared with the non-AKI group

not rise earlier than sCr. On the 2 days prior to AKI, uCyC levels did not differ significantly between group 1 and group 0 (median 0.13 [0.025–0.88] mg/L versus median 0.16 [0.025–1.50] mg/L, $P = 0.89$, and median 0.14 [0.025–0.37] mg/L versus median 0.17 [0.025–0.84] mg/L, $P = 0.57$, Day –2 and Day –1, respectively). The
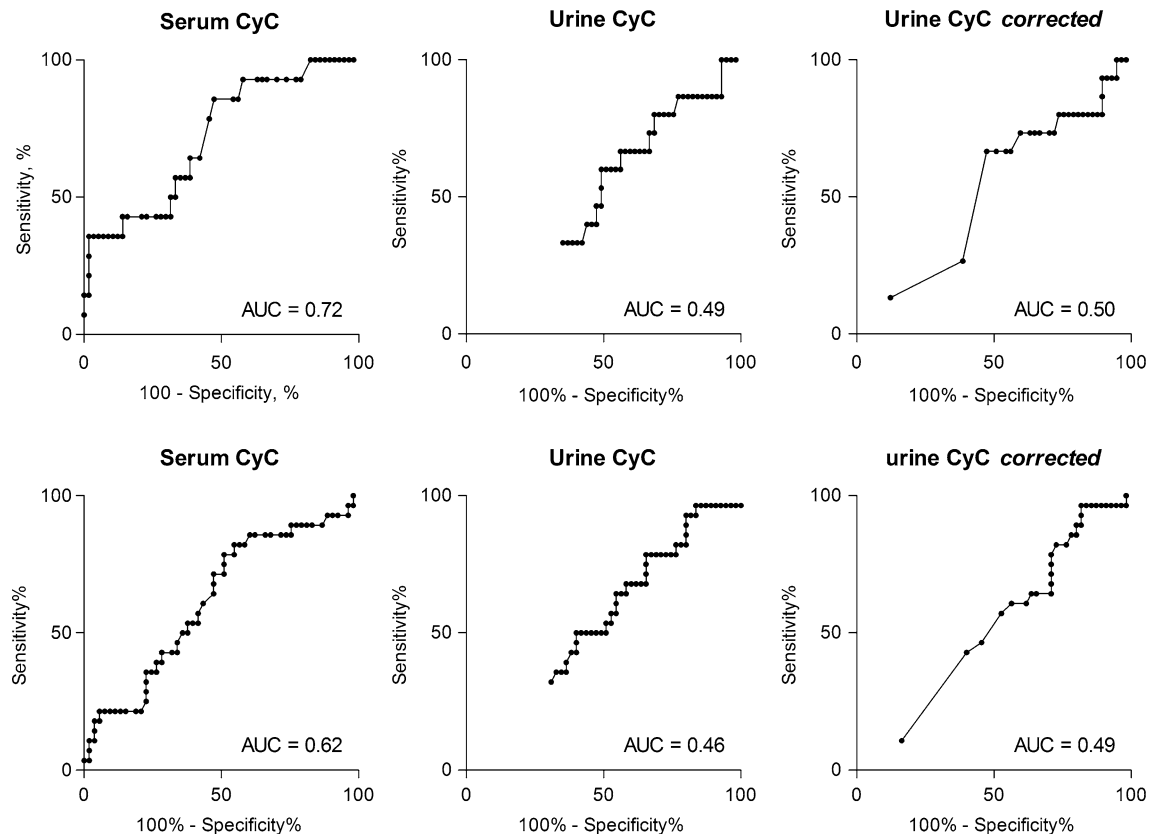
Fig. 2 Receiver-operating curves demonstrating the performance of cystatin C (CyC) for prediction of acute kidney injury (AKI). *Upper panels* 2 days prior to AKI ($N = 71$, disease prevalence 0.20); *lower panels* 1 day prior to AKI. ($N = 81$, disease prevalence 0.35). Area under the curve (AUC) is depicted in each panel. $uCyC_{corr}$ urine cystatin C normalized for urinary creatinine

diagnostic performance for sCyC was fair on Day −2 [area under the curve (AUC) 0.72] and poor on Day −1 (AUC 0.62), while uCyC had no diagnostic value on Day −2 and Day −1 (Fig. 2). The test characteristics of sCyC at various cutoff levels are shown in ESM Table 1.

Fourteen (15%) out of 91 AKI patients received continuous venovenous hemofiltration (CVVH): 4 patients from group 1, and 10 patients from group 2. Median duration between Day 0 and initiation of CVVH was 1.5 [0–4] days. In comparison with the non-CVVH patients, the AKI patients requiring CVVH had significantly higher APACHE II scores on admission (28 [21–33] versus 19 [14–27], $P = 0.01$) and produced less urine (740 [400–1,088] mL/day versus 1,660 [1,028–2,918] mL/day, $P = 0.001$). Systemic and urinary CyC determined on Day 0 were poor predictors for the need for RRT (AUC ≤ 0.66, Fig. 3).

Discussion

In this prospective multicenter cohort study in a heterogeneous ICU population, sCyC and uCyC increased with increasing RIFLE class. However, the predictive ability of these biomarkers for AKI was poor. Approximately one-third of the patients without AKI at ICU admission developed AKI during the ICU treatment, and in these patients sCyC was significantly higher 2 days prior to AKI compared with patients who did not develop AKI. Of note, sCyC levels did not rise earlier than sCr. The diagnostic performance of sCyC was at best fair for Day −2 and poor on Day −1. Levels of uCyC were not different in patients prior to AKI in comparison with patients who did not develop AKI and had no diagnostic accuracy for AKI.

Previous studies investigating whether CyC increases before development of AKI are limited and their results inconsistent; however, comparison among studies is hampered by case mix and heterogeneity in study designs [7, 12, 20–24]. Notably, no two studies used identical definitions for AKI, and this definition is critical in biomarker research. Nejat et al. [20, 25, 26] recently reported on the predictive performance of CyC in two subanalyses of the EARLYARF study. Notably, while 3,966 patients were screened for the EARLYARF study, only 444 patients (11%) were included in the subanalyses, suggesting selection bias [25]. Based on the sCr criteria used
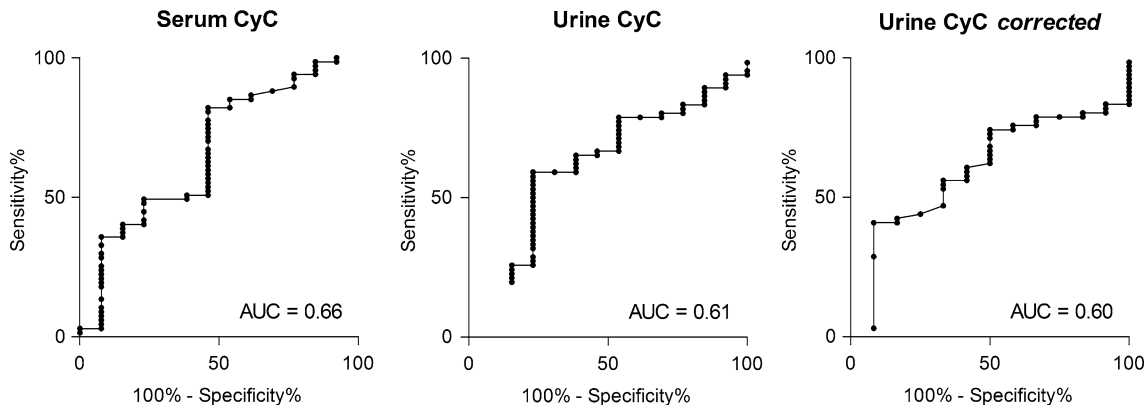
Fig. 3 Receiver-operating curves demonstrating the performance of cystatin C (CyC) for prediction of the need of renal replacement therapy. ($N = 80$, disease prevalence 0.16). Area under the curve (AUC) is depicted in each panel

by the Acute Kidney Injury Network definition [27], 125 (28%) patients had AKI on entry, 73 (16%) patients developed AKI over the subsequent 7 days (AKI 7d), and 246 (55%) patients did not [20, 26]. sCyC rose prior to sCr in 66% of the patients developing AKI after entry [20]. sCyC on entry was predictive for sustained AKI (AUC 0.80), defined as an increase in sCr of at least 50% from baseline for 24 h or longer, but not for AKI 7d (AUC 0.65) [20]. A subanalysis of sepsis patients showed that only uCyC was predictive of AKI within 48 h (AUC 0.71) and not sCyS, while uCyC was not predictive of AKI in patients without sepsis (AUC = 0.45) [26]. Ahlström et al. [21] studied 202 patients in a mixed ICU, of whom 49 developed AKI according to the urine output and/or sCr RIFLE failure criteria. In that study sCyC showed excellent predictive value for AKI; however, sCyC did not rise earlier than sCr [21]. Herget-Rosenthal et al. [7] analyzed 85 ICU patients at high risk of developing AKI and used the creatinine risk criteria of the RIFLE classification to define AKI ($N = 44$). In contrast to our findings, sCyC was shown to detect AKI 1–2 days earlier than sCr (AUC 0.82 and 0.97 on day –2 and day –1, respectively) [7]. In the study by Haase-Fielitz et al. [23] in 100 patients following cardiopulmonary bypass surgery (CPB), 23 patients developed AKI, defined as increase in sCr of $\geq 50\%$ from baseline within the first five postoperative days. In that study, sCyC on ICU arrival and at 24 h after CPB were good predictors of AKI (AUC ≈ 0.83). Notably, the authors suggested that presence of chronic kidney disease (CKD) could affect the diagnostic performance of CyC, because exclusion of the CKD patients reduced the diagnostic performance of sCyC. This reduction in diagnostic performance, however, may have been caused by the smaller sample size ($N = 15$), rather than by the exclusion of CKD patients [23]. We did not evaluate the effect of CKD, because only one patient had CKD on day –2 and day –1. In another, small ($N = 30$) CPB study, 15 patients developed AKI defined as 50% or greater increase in sCr from baseline

within 72 h, and the optimal diagnostic performance for sCyC (AUC 0.83) was 10 h post CPB [22]. In the CPB study ($N = 72$) of Koyner, 34 patients developed AKI defined as sCr rise of $\geq 25\%$ from baseline within the first 3 days or need of RRT [12]. Serum CyC at ICU arrival and at 6 h were poor predictors for AKI (AUC 0.62 and 0.63, respectively), while urine CyC at ICU arrival and at 6 h were fair predictors (AUC 0.69 and 0.72, respectively) [12]. In the study by Liangos et al. [24] ($N = 103$), uCyC measured 2 h post CPB did not predict AKI ($N = 13$), defined as sCr increase of $\geq 50\%$ from post CPB to peak value within the first 3 days (AUC 0.50). In a very small study in septic patients ($N = 29$) no correlation was found between sCyC at ICU admission and occurrence of AKI ($N = 10$) defined as sCr >267 μmol/L or diuresis <30 mL/h [28]. Four small studies in ICU patients reported that sCyC was superior to sCr to identify glomerular filtration rate (GFR) <80 mL/min per 1.73 m^2 [29–32]. The GFR studies, however, can be criticized because they used derivatives for GFR and not the real "gold standard" based on clearance of exogenous substances [33].

In the present study, 56 (37%) patients had AKI at admission, which is similar to a previous AKI biomarker study in a heterogeneous ICU population [34]. It make little sense to predict AKI in patients with AKI; however, in these patients, CyC may help to identify those requiring RRT. Unfortunately, in our study both sCyC and uCyC determined on day 0 were poor predictors for the need for RRT, although it must be mentioned that RRT was started in 14 patients only. Our findings agree with the findings of Perianayagam et al. [35], but disagree with three earlier reports suggesting excellent diagnostic performance of sCyC [11, 20, 23]. Several factors may explain the conflicting results, including case mix and the reason for starting RRT. In our study, oligo/anuria often triggered RRT, while low GFR probably triggered RRT in the study by Herget-Rosenthal et al., including exclusively nonoliguric patients [11]. In the study by Haase-Fielitz et al. only five patients fulfilled the composite endpoint (need for RRT and in-hospital mortality) [23]. Nejat et al. do not report how many patients received RRT [20].

Another potential role for biomarkers is to allow differentiation between extrinsic and intrinsic causes of AKI; however, our study was not designed to investigate this issue.

Nearly 40% of our patients never developed AKI, yet some of these patients had increased levels of CyC according to the reference intervals reported in literature [10]. Several factors may be responsible for this finding. First, the published reference intervals were determined in healthy volunteers with no history of renal disease, and may not apply to our critically ill population. Although sCyC is less influenced by age, sex, and muscle mass compared with serum creatinine level, it still can be affected by these patient variables [36]. Moreover, sCyC levels can be affected by levels of glucocorticosteroids [37], thyroid hormones [38], and insulin [39] as well as markers of inflammation such as white blood cell count and C–reactive protein level [40]. All these factors play an important role in critically ill patients and could affect the reference interval. Second, we did not use any protease inhibitor in the storage of the urine to clear all disturbing elements in the urine. However, Herget-Rosenthal reported good agreement between uCyC measurement with and without stabilization buffer [10]. Finally, the consensus RIFLE definition for AKI is based on sCr and/or urine output. These are functional parameters and may not be appropriate for detection of injury to the kidney. We cannot rule out that increased CyC levels indeed reflect damage to the kidneys which is not recognized by the RIFLE criteria. Notably, in the present study, prevalence of AKI was high, and therefore a low sCyC of <0.80 mg/L resulted in high [negative predictive value (NPV) 75–95%] despite its low specificity. Therefore, in a critically ill population with high prevalence of AKI, sCyC may be helpful to rule out AKI.

Our study is the first multicenter study investigating whether CyC can predict development of AKI in the general critical care setting in which the population is heterogeneous and AKI etiology and timing are often unclear. However, there are some limitations. First, a small percentage of our patients (12%) were enrolled the day following admission, and specimens were collected on alternate days after the first 2 days. As a result, our sample size was relatively small, particularly on Day –2. Second, MDRD-based baseline sCr was used in 18% of our patients. Missing preadmission sCr value is a recognized problem in AKI research, which may lead to misclassification of AKI incidence [41, 42]. On the other hand, use of surrogate measures for baseline renal function avoids selection bias. Third, we did not evaluate whether CyC concentrations were affected by other factors (e.g., inflammatory state, insulin or hydrocortisone therapy). Finally, the definition of AKI is critical in biomarker research, and the RIFLE criteria may not be an adequate gold standard for AKI.

Conclusions

In this relatively small multicenter study in a heterogeneous ICU population and using the RIFLE classification system to define AKI, sCyC and uCyC were poor biomarkers for AKI. Moreover, on the first day of AKI these biomarkers did not predict the need of RRT. However, sCyC <0.80 mg/L had high NPV in our population with high incidence of AKI.

References

1. Joannidis M, Metnitz PG (2005) Epidemiology and natural history of acute renal failure in the ICU. Crit Care Clin 21:239–249
2. Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M, Tan I, Bouman C, Macedo E, Gibney N, Tolwani A, Oudemans-van SH, Ronco C, Kellum JA (2007) Continuous renal replacement therapy: a worldwide practice survey. The beginning and ending supportive therapy for the kidney (B.E.S.T. kidney) investigators. Intensive Care Med 33:1563–1570. doi:10.1007/s00134-007-0754-4
3. Kolhe NV, Stevens PE, Crowe AV, Lipkin GW, Harrison DA (2008) Case mix, outcome and activity for patients with severe acute kidney injury during the first 24 hours after admission to an adult, general critical care unit: application of predictive models from a secondary analysis of the ICNARC Case Mix Programme database. Crit Care 12(Suppl 1):S2. doi:10.1186/ccforum.com/content/12/S1/S2
4. Jo SK, Rosner MH, Okusa MD (2007) Pharmacologic treatment of acute kidney injury: why drugs haven't worked and what is on the horizon. Clin J Am Soc Nephrol 2:356–365. doi:10.2215/CJN.03280906
5. Bouman CS, Forni LG, Joannidis M (2010) Biomarkers and acute kidney injury: dining with the Fisher King? Intensive Care Med 36:381–384. doi:10.1007/s00134-009-1733-8

6. Parikh CR, Devarajan P (2008) New biomarkers of acute kidney injury. Crit Care Med 36:S159–S165. doi: 10.1097/CCM.0b013e318168c652

7. Herget-Rosenthal S, Marggraf G, Husing J, Goring F, Pietruck F, Janssen O, Philipp T, Kribben A (2004) Early detection of acute renal failure by serum cystatin C. Kidney Int 66:1115–1122

8. Conti M, Zater M, Lallali K, Durrbach A, Moutereau S, Manivet P, Eschwege P, Loric S (2005) Absence of circadian variations in urine cystatin C allows its use on urinary samples. Clin Chem 51:272–273. doi: 10.1373/clinchem.2004.039123

9. Conti M, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, Eschwege P, Loric S (2006) Urinary cystatin C as a specific marker of tubular dysfunction. Clin Chem Lab Med 44:288–291. doi: 10.1515/CCLM.2006.050

10. Herget-Rosenthal S, Feldkamp T, Volbracht L, Kribben A (2004) Measurement of urinary cystatin C by particle-enhanced nephelometric immunoassay: precision, interferences, stability and reference range. Ann Clin Biochem 41:111–118

11. Herget-Rosenthal S, Poppen D, Husing J, Marggraf G, Pietruck F, Jakob HG, Philipp T, Kribben A (2004) Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 50:552–558. doi: 10.1373/clinchem.2003.027763

12. Koyner JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V, Kasza KE, O'Connor MF, Konczal DJ, Trevino S, Devarajan P, Murray PT (2008) Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 74:1059–1069. doi: 10.1038/ki.2008.341

13. Uchida K, Gotoh A (2002) Measurement of cystatin-C and creatinine in urine. Clin Chim Acta 323:121–128

14. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P (2004) Acute renal failure—definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 8:R204–R212. doi:10.1186/cc2872

15. Bouman CSC, Royakkers AA, Korevaar JC, van Suijlen JD, Hofstra LS, Kuiper MA, Spronk PE, Schultz MJ (2009) The utility of urinary cystatin C as early predictive biomarkers for acute kidney injury in critically il patients admitted to the ICU. Intensive Care Med 35:S221

16. Knaus WA, Draper EA, Wagner DP, Zimmerman JE (1985) APACHE II: a severity of disease classification system. Crit Care Med 13:818–829

17. Le Gall Jr, Lemeshow S, Saulnier F (1993) A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 270:2957–2963

18. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130:461–470

19. Morgenstern BZ, Butani L, Wollan P, Wilson DM, Larson TS (2003) Validity of protein-osmolality versus protein-creatinine ratios in the estimation of quantitative proteinuria from random samples of urine in children. Am J Kidney Dis 41:760–766

20. Nejat M, Pickering JW, Walker RJ, Endre ZH (2010) Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit. Nephrol Dial Transplant doi: 10.1093/ndt/gfq176

21. Ahlstrom A, Tallgren M, Peltonen S, Pettila V (2004) Evolution and predictive power of serum cystatin C in acute renal failure. Clin Nephrol 62:344–350

22. Che M, Xie B, Xue S, Dai H, Qian J, Ni Z, Axelsson J, Yan Y (2010) Clinical usefulness of novel biomarkers for the detection of acute kidney injury following elective cardiac surgery. Nephron Clin Pract 115:c66–c72. doi: 10.1159/000286352

23. Haase-Fielitz A, Bellomo R, Devarajan P, Story D, Matalanis G, Dragun D, Haase M (2009) Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. Crit Care Med 37:553–560. doi: 10.1097/CCM.0b013e318195846e

24. Liangos O, Tighiouart H, Perianayagam MC, Kolyada A, Han WK, Wald R, Bonventre JV, Jaber BL (2009) Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. Biomarkers 14:423–431. doi: 10.1080/13547500903067744

25. Endre ZH, Walker RJ, Pickering JW, Shaw GM, Frampton CM, Henderson SJ, Hutchison R, Mehrtens JE, Robinson JM, Schollum JB, Westhuyzen J, Celi LA, McGinley RJ, Campbell IJ, George PM (2010) Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial). Kidney Int 77:1020–1030. doi:10:10.1038/ki.2010.25

26. Nejat M, Pickering JW, Walker RJ, Westhuyzen J, Shaw GM, Frampton CM, Endre ZH (2010) Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. Crit Care 14:R85

27. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A (2007) Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 11:R31. doi: 10.1186/cc5713

28. Mazul-Sunko B, Zarkovic N, Vrkic N, Antoljak N, Bekavac BM, Nikolic HV, Siranovic M, Krizmanic-Dekanic A, Klinger R (2004) Proatrial natriuretic peptide (1–98), but not cystatin C, is predictive for occurrence of acute renal insufficiency in critically ill septic patients. Nephron Clin Pract 97:c103–c107

29. Delanaye P, Lambermont B, Chapelle JP, Gielen J, Gerard P, Rorive G (2004) Plasmatic cystatin C for the estimation of glomerular filtration rate in intensive care units. Intensive Care Med 30:980–983. doi: 10.1007/s00134-004-2189-5

30. Le Bricon T, Leblanc I, Benlakehal M, Gay-Bellile C, Erlich D, Boudaoud S (2005) Evaluation of renal function in intensive care: plasma cystatin C vs. creatinine and derived glomerular filtration rate estimates. Clin Chem Lab Med 43:953–957. doi: 10.1515/CCLM.2005.163

31. Villa P, Jimenez M, Soriano MC, Manzanares J, Casasnovas P (2005) Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. Crit Care 9:R139–R143. doi:10.1186/cc3044

32. Herrero-Morin JD, Malaga S, Fernandez N, Rey C, Dieguez MA, Solis G, Concha A, Medina A (2007) Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children. Crit Care 11:R59. doi:10.1186/cc5923

33. Wulkan R, den HJ, Berghout A (2005) Cystatin C: unsuited to use as a marker of kidney function in the intensive care unit. Crit Care 9:531–532. doi: 10.1186/cc3541

34. Cruz DN, de CM, Garzotto F, Perazella MA, Lentini P, Corradi V, Piccinni P, Ronco C (2010) Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med 36:444–451. doi: 10.1007//00134-009-1711-1

35. Perianayagam MC, Seabra VF, Tighiouart H, Liangos O, Jaber BL (2009) Serum cystatin C for prediction of dialysis requirement or death in acute kidney injury: a comparative study. Am J Kidney Dis 54:1025–1033

36. Groesbeck D, Kottgen A, Parekh R, Selvin E, Schwartz GJ, Coresh J, Furth S (2008) Age, gender, and race effects on cystatin C levels in US adolescents. Clin J Am Soc Nephrol 3:1777–1785

37. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B (2002) Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. Clin Chem 48:1123–1126

38. Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C (2003) Impact of thyroid dysfunction on serum cystatin C. Kidney Int 63:1944–1947

39. Yokoyama H, Inoue T, Node K (2009) Effect of insulin-unstimulated diabetic therapy with miglitol on serum cystatin C level and its clinical significance. Diabetes Res Clin Pract 83:77–82

40. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, Froissart M, Kusek JW, Zhang YL, Coresh J, Levey AS (2009) Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int 75:652–660. doi:10.1038/ki.2008.638

41. Joannidis M, Metnitz B, Bauer P, Schusterschitz N, Moreno R, Druml W, Metnitz PG (2009) Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. Intensive Care Med 35:1692–1702. doi:10.1007/s00134-009-1530-4

42. Siew ED, Matheny ME, Ikizler TA, Lewis JB, Miller RA, Waitman LR, Go AS, Parikh CR, Peterson JF (2010) Commonly used surrogates for baseline renal function affect the classification and prognosis of acute kidney injury. Kidney Int 77:536–542. doi:10.1038/ki.2009.479