Impact of sepsis on levels of plasma cystatin C in AKI and non-AKI patients

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Abstract

Background. Cystatin C is a marker of acute kidney injury (AKI). However, systemic inflammation associated with sepsis, a common AKI-trigger, may affect cystatin C. We studied the impact of sepsis on cystatin C levels in plasma. Furthermore, we investigated whether the presence of sepsis affects the predictive properties of cystatin C.

Methods. Three hundred and twenty-seven intensive care unit (ICU) patients were categorized as having: neither AKI nor sepsis (n = 151), sepsis without AKI (n = 80), AKI without sepsis (n = 24) or AKI and sepsis (n = 72) during their first week in the ICU. Changes in cystatin C and creatinine over time in patients with and without sepsis or AKI were analysed using repeated measures analysis of variance. The performance of cystatin C on admission to predict sustained AKI, worsened AKI or death was assessed from the area under the receiver-operating characteristic curve (AUC-ROC) in septic and non-septic patients separately.

Results. In non-AKI patients, cystatin C increased and creatinine decreased significantly over the first week. The change in cystatin C or creatinine did not differ significantly between septic and non-septic patients without AKI. Even in AKI patients, the cystatin C change did not differ significantly between septic and non-septic patients. The AUC-ROCs for prediction of the composite outcome were 0.80 and 0.78 in patients with and without sepsis, respectively, and did not differ significantly (P = 0.76).

Conclusion. The inflammatory response induced by sepsis has no impact on the levels of cystatin C in plasma during the first week in the ICU.

Keywords: acute kidney injury; acute renal failure; biomarkers; creatinine; cystatin C

Introduction

Acute kidney injury (AKI) is a common complication in intensive care unit (ICU) patients and is associated with high mortality, prolonged length of ICU stay and increased post-hospital morbidity [1, 2]. Early diagnosis of AKI may significantly affect our ability to improve the clinical course of the disease.

While many new markers of kidney injury are under study, the use of functional data, like urine output and plasma creatinine, dominates clinical reality. The shortcomings of plasma creatinine as an indicator for deteriorating kidney function have been extensively documented [3]. In a number of studies, cystatin C, another functional biomarker, has been found to be superior to creatinine in detecting minor reductions in glomerular filtration rate (GFR) [4–6]. However, the practical use of cystatin C in general ICU patients are still under debate. In two studies on mixed ICU patients, cystatin performed well as an AKI predictor [7, 8]. In contrast, Royakkers et al. [9] recently found serum cystatin C to be a poor predictor of AKI. The performance of cystatin C as a predictor of dialysis and death has, at best, been moderate [8, 10]. Recent studies have found an association between increased cystatin C levels and systemic inflammation and sepsis [11–14]. This might cause a diagnostic problem in the general ICU setting where sepsis is abundant and at the same time has been identified as the major cause of AKI [15].

In this study, we investigated the potential impact of sepsis on cystatin C levels in AKI and non-AKI patients. In addition, we studied if the performance of cystatin C to predict sustained AKI, worsened AKI or mortality was affected by the presence of sepsis.

Materials and methods

This study was approved by the regional ethical review board in Stockholm.

Patients were recruited from two parallel ongoing studies at our department. One is a prospective outcome study on trauma patients admitted to our ICU and the other is a prospective study on renal biomarkers in patients with and without AKI. In total, 368 patients were enrolled in these two studies from February 2007 until April 2010. We identified patients who were enrolled in both studies so that no single patient was recorded twice.

Blood samples were collected and patients were, as part of routine care, weighed on admission to the ICU and thereafter once daily at 6 am until discharge from the ICU. Samples were immediately analysed for plasma cystatin C, plasma creatinine and C-reactive protein (CRP) at the Department of Clinical Chemistry, Karolinska University Hospital Solna.

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Cystatin C was measured by the Gentian immunoassay (Gentian, Moss, Norway). The total analytic imprecision was 7% at 1.0 mg/L and 3.9% at 3.4 mg/L. CRP was measured by latex-enhanced turbidimetry (coefficient of variation 4–5%) and creatinine was measured by alkaline picrate colorimetry (coefficient of variation 4–9%). Analyses were performed on the LX/Dx C 800 analyzer (Beckman-Coulter, CA).

Patients were screened daily for the presence of AKI and/or sepsis. AKI was defined as a rise in plasma creatinine 50% relative baseline (delta creatinine) [16]. If present, we included creatinine values obtained within 48 h before and after ICU stay for the AKI classification. The lowest creatinine level found within 3 months prior to ICU admission was used as baseline value. When pre-admission creatinine was lacking, baseline creatinine was estimated by the modification of diet in renal disease equation based on a GFR of 75 ml/min/1.73m². AKI duration was defined as the time frame from the first until the last delta creatinine 50%. The sepsis classification was in accordance with the definitions suggested by the American College of Chest Physicians/Society of Critical Care Medicine with modification of the systemic inflammatory response (SIRS) criteria applied by the Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group [17, 18].

Patients were grouped into one of four categories according to the presence of AKI and sepsis during the first 7 days: Category A, neither AKI nor sepsis; Category B, sepsis without AKI; Category C, AKI without sepsis and Category D, sepsis and AKI. In the analysis, we only included values for cystatin C, creatinine and body weight on ICU days when the pre-defined criteria for each group were fulfilled. For example, in Category D, we only included data on days when AKI and sepsis were present simultaneously. For patients in Category C, we included data only on ICU days when these patients suffered from AKI. Patients in Category B only contributed with data when they fulfilled the criteria for sepsis. In Category A, data on all 7 days (if present) were included since these patients neither had sepsis nor AKI at any time during the time frame of 48 h before until 48 h after ICU stay. Biomarker levels and weight recordings obtained during renal replacement therapy (RRT) were excluded.

Data were analysed using STATA® for Windows version 10.1 software (StataCorp, College Station, TX). Continuous variables were expressed as median and interquartile range and categorical variables as n (%). The Mann–Whitney U-test was used for comparison between continuous variables and the χ²-test or the Fisher’s exact test for comparisons between categorical variables. The changeover time for cystatin C, creatinine, CRP and body weight was tested by a repeated measure analysis of variance (ANOVA) after logarithmic transformation (base 10). For comparison of changeover time between categories, an interaction variable (between category and time) was introduced in the ANOVA model. Diagnostic characteristics of plasma cystatin C were assessed by receiver-operating characteristic analysis. Areas under the receiver-operating characteristic curves (AUC-ROC) were compared by the χ²-test. A two-sided P-value < 0.05 was considered statistically significant.

Results

Of 368 patients, 151 had neither AKI nor sepsis (Category A), 80 had sepsis without AKI (Category B), 24 had AKI without sepsis (Category C) and 72 had concomitant AKI and sepsis (Category D) during the first week. 41 patients were excluded since AKI and sepsis did not occur simultaneously, AKI or sepsis occurred after 7 days in the ICU or cystatin C values were not obtained. Baseline characteristics, ICU course during the first week and outcomes for patients included in the four different categories are shown in Table 1. Note that one patient in the no AKI/no sepsis group (Category A) had sepsis as admission diagnosis. This patient, however, never fulfilled the SIRS criteria.

Acute physiology and chronic health evaluation II scores as well as creatinine, delta creatinine and cystatin C levels on admission were significantly higher in septic patients without AKI as compared to their non-septic counterparts. In the AKI patients with and without sepsis, these measures did not differ significantly.

Over the first 7 days, no significant difference in median or peak delta creatinine between septic and non-septic patients was found. This was true both for AKI and non-AKI patients. However, septic patients had higher median and peak cystatin C levels during the first week as compared to non-septic patients.

Highlighting outcome, septic patients stayed three to four times longer in the ICU as compared to non-septic patients. ICU- and 30-day-mortality did not differ significantly in septic as compared to non-septic patients.

Changes of cystatin C, creatinine and body weight over time in non-AKI patients

Non-AKI patients with sepsis had a slightly worse kidney function on admission than their non-septic counterparts indicated by higher levels of creatinine, delta creatinine and cystatin C levels. In the non-AKI patients, cystatin C increased during the first week in the ICU. After 7 days, the mean increase of cystatin C was 24 ± 19% (mean ± SD) in the non-septic patients (Figure 1A) and 37 ± 40% in the septic patients (Figure 1B). Changes were significant for the non-septic (ANOVA; P < 0.0001) and septic (ANOVA; P = 0.0001) patients during this time frame. No significant difference in cystatin C changeover time was found between septic and non-septic patients (interaction; P = 0.59). Contrasting the cystatin C-change, creatinine decreased significantly over time in both the non-septic (ANOVA; P < 0.0001; Figure 1A) and septic (ANOVA; P < 0.0001; Figure 1B) patients without AKI. As with cystatin C, changes in creatinine did not differ significantly between septic and non-septic patients (interaction; P = 0.72). To study the impact of variations in total body water (TBW) on biomarker levels, the corresponding changes in body weight over the first week were also included in Figure 1. We thereby assumed that daily alterations in body weight mainly reflected corresponding changes in TBW. Body weight initially increased reaching 1.4 ± 3.6% on Day 5 in non-septic patients and 0.8 ± 5.7% on Day 3 in septic patients. Body weight decreased below admission values thereafter. Body weight changes were only significant in septic patients (ANOVA; P < 0.0001).

Changes of cystatin C, creatinine and body weight over time in AKI patients

In the AKI patients, cystatin C continued to increase after ICU admission both in the absence (Figure 1C) and presence (Figure 1D) of sepsis. Non-septic patients were only analysed until Day 5 since values for cystatin C were lacking after that time point. Over this time frame, no significant change in cystatin C was observed (ANOVA; P = 0.65). In the septic patients, a significant increase in cystatin C was found during the first week (ANOVA; P < 0.0001). We found no significant difference when comparing the cystatin C time courses between septic and non-septic patients over the first 5 days (interaction; P = 0.13). However, the limited power available from the small sample of patients in the non-septic AKI cohort must be acknowledged. Creatinine did not change significantly over the first week in the non-septic (ANOVA; P = 0.35) or the septic
In septic patients, CRP decreased slightly but significantly (ANOVA; P = 0.0001) over the first week. In the non-septic cohort, CRP increased dramatically during the first 48 h. Apart from ICU Days 6 and 7, CRP levels were significantly higher in septic patients as compared to non-septic patients on each day. No significant correlation was found when comparing cystatin and CRP levels on each separate ICU day. This was true both for the septic and the non-septic patients (Table 2).

Cystatin C on admission as a predictor of sustained AKI, worsening AKI or death within 7 days in septic versus non-septic patients

The impact of sepsis on the property of cystatin C on admission to predict sustained AKI (>3 days), worsening AKI (defined as an increase in RIFLE [Risk, Injury, Failure, Loss, End-stage renal disease] stage or RRT initiation)
or death within 7 days was analysed. Of the 368 patients, 90 (24%) had AKI on admission. Of these 90 patients, 40 (44%) either maintained their creatinine levels ≥50% relative baseline for >3 days, increased their RIFLE score, were started on RRT or died during the first week. Of 278 patients without AKI on admission, 36 (13%) patients developed AKI and seven (2.5%) patients died within the first 7 days. Of 332 patients with existing values of cystatin C on admission, 77 fulfilled the criteria for the composite outcome. Of these 77 patients, 30 (39%) were classified as having sepsis on admission. Of the remaining 255 patients without the composite outcome, 37 (15%) had sepsis on admission.

Performance of cystatin C on admission as a predictor of the composite outcome is shown in Figure 3 for non-septic and septic patients, respectively. The AUC-ROC for non-septic patients was 0.78 (95% CI 0.70–0.85) and the corresponding AUC-ROC for septic patients was 0.80 (95% CI 0.68–0.91). There was no significant difference between the two AUC-ROCs (χ²-test; P = 0.76).

Discussion

In this single centre study, sepsis per se did not affect cystatin C levels in plasma. Previous studies have considered the systemic inflammation caused by sepsis as a possible explanation for elevated cystatin C in plasma and its
impact on mortality [10, 13, 19]. In a recent study, we found a stepwise increase in peak levels of plasma cystatin C with increasing sepsis severity in ICU patients without AKI [13]. In the present investigation, both median and peak levels of cystatin C were significantly higher in septic as compared to non-septic patients during the first week irrespective of whether AKI was present or not. A longer ICU length of stay in septic patients can explain this finding. In fact, when we displayed the time course over the first week, we found a gradual increase in cystatin C over time in all four study groups. Moreover, we found no significant difference when comparing this increase over time between septic and non-septic patients. Also, the gradual decrease in creatinine over the first week was similar in septic and non-septic patients. Notably, known confounders such as age and body weight did not differ between septic and non-septic patients (Table 1). The inflammatory response induced by sepsis is probably more severe than the SIRS seen in many non-septic ICU patients. To investigate this, we compared CRP levels between the septic and non-septic cohorts. Both the median and peak CRP levels were significantly higher in the septic as compared to the non-septic patients. Although CRP levels gradually increased in the non-septic patients, their levels were significantly lower on each of the first 5 days in the ICU as compared to the corresponding levels in the septic patients. This supports that the inflammatory response was more severe in the septic cohort. The lack of influence of sepsis on cystatin C was supported by our additional findings (i) that no correlation was found between CRP and cystatin C on any of the first 7 days in the ICU (Table 2) and (ii) that cystatin C performed equally well as an on admission predictor of sustained AKI, worsening AKI, dialysis or death regardless of whether the patients were septic or not (Figure 3).

Our findings are in line with those of Grubb et al. [20], who conducted a study not supporting a causal relationship between inflammation post-surgery and levels of cystatin C. The data from Grubb et al., along with our findings, question the correlation of CRP as a measure of inflammation and cystatin C found in two large cross-sectional studies [11, 12]. Following tissue damage, proteolytic enzymes (e.g. cysteine proteases) are released [21, 22]. Cystatin C, a potent protease inhibitor, might be consumed during such conditions. This may blunt a potential rise in cystatin C levels induced by the inflammatory response and hence affect the results found by Grubb et al. and by us. The majority (91%) of patients without sepsis or AKI in our study were admitted following severe traumatic injury. Despite the extensive tissue damage in this population, cystatin C levels gradually increased. This indicates that cystatin C consumption, if present, only affects plasma levels marginally.

In our non-AKI patients, cystatin C rises and creatinine falls significantly over time. In fact, in a recent study, Nejat et al. [8] found almost the same temporal decrease of creatinine. A possible explanation for this is the loss of muscle mass due to immobilization [23] and/or catabolism well known to occur in ICU patients [24]. Theoretically, a fall in creatinine can also be explained by dilution due to increase in TBW [25]. However, this is unlikely to explain the decline in creatinine in this study since body weight, our proxy for TBW, did not change significantly.

A gradual decline in creatinine in critically ill patients may hamper the use of creatinine as a GFR marker in this setting. In our AKI cohort, we do see unchanged levels of creatinine (Figure 1), whereas cystatin C levels rise, almost doubling by Day 7. Whether cystatin C, a larger molecule than creatinine, is an earlier marker of decreasing GFR during critical illness remains to be seen. Small reductions in glomerular pore size still allow free filtration of creatinine, whereas filtration of larger molecules like cystatin C may be impaired.

The rise of cystatin C may, however, be explained by non-renal factors. Use of corticosteroids, at least in high doses, could affect cystatin C levels [26–28]. In this study, a more frequent use of steroids in septic as compared to non-septic patients was not accompanied by a more pronounced increase in cystatin C. Furthermore, it is unlikely that the few patients on high-dose steroids caused the different changes of creatinine and cystatin C over time. Finally, the catabolic state per se might cause an up-regulation of cystatin C due to rapid cell turnover, although we have not been able to find studies supporting this hypothesis.

Unique to this study is the detailed information on the day-by-day incidence of AKI and/or sepsis. By studying the functional biomarkers cystatin C and creatinine only when patients did or did not fulfil sepsis and/or AKI criteria, we were able to evaluate the impact of these conditions on the respective biomarker levels. This study has limitations. Firstly, we have no gold standard day-to-day-measurements of GFR. It could be possible to determine whether cystatin C is a better GFR determinant than creatinine during the course of critical illness. This theoretical study would entail daily measurements of the two biomarkers combined with daily gold-standard (using, for example, iohexol or inulin) GFR measurements, as well as day-to-day data of fluid balance and body weight. Secondly, our sample size in the non-septic AKI group was small, resulting in lack of discrimination of non-septic and septic AKI. Thirdly, to study the influence of TBW changes...
on biomarker levels, repeated measurements of TBW changes by isotope dilution techniques would have been preferred. Finally, the AKI definition used in this study relies on changes in plasma creatinine relative baseline. Since a true baseline value was lacking in a significant number of patients, especially in the AKI cohorts, misclassification might be a problem. Nevertheless, the comparisons made between septic and non-septic patients are still valid since the proportions of available baseline values were equal among patients with and without sepsis.

Conclusions

Sepsis per se does not affect levels of cystatin C. Creatinine is still the dominant functional marker used by intensivists worldwide. However, as creatinine levels decrease over time, it should be used with caution, especially late in the clinical course of the ICU patient. Since cystatin C seems to be unaffected by several non-renal factors affecting creatinine levels, cystatin C might have benefits over creatinine as an online GFR marker in the ICU.

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