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Comparison of serum cystatin C and creatinine changes after cardiopulmonary bypass in patients with normal preoperative kidney function

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Running head: Changes in kidney function biomarkers after heart surgery

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Abstract

Purpose: Serum creatinine is used ubiquitously to estimate glomerular filtration rate and to diagnose acute kidney injury (AKI) after cardiac surgery. Serum cystatin C is a novel biomarker that has emerged as a possible diagnostic alternative to serum creatinine. It is unclear if the dynamic changes in serum cystatin C immediately following cardiopulmonary bypass (CPB) differ from those of serum creatinine in patients with normal preoperative kidney function.

Methods: We compared changes in serum levels of creatinine and cystatin C by measuring them serially in 19 patients undergoing CPB. Within-patient differences for serum creatinine and serum cystatin C were compared by repeated measures ANOVA.

Results: Serum creatinine and cystatin C levels showed significant correlation with each other. Both biomarkers showed a significant decrease after CPB, but their serum concentrations reverted to pre-CPB levels within 12 hours. Serum levels of serum creatinine remained unchanged from baseline levels throughout 72 hours post-CPB. In contrast, serum cystatin C levels rose further, and became significantly higher compared to baseline within 48 hours. Serum cystatin C remained significantly elevated at 48 and 72 hours post-CPB.

Conclusions: Processes that determine the serum concentrations of serum creatinine and cystatin C in the post-CPB period affect the two biomarkers differently, suggesting that the two are not interchangeable as diagnostic markers of glomerular filtration rate. Future studies are needed to examine if these discrepancies are related to differences in their production rates, in their ability to detect small changes in GFR, or to a combination of these, and to determine the effect of such differences on the diagnostic and prognostic accuracy of the two biomarkers.

Key words: cardiopulmonary bypass, glomerular filtration rate, creatinine, cystatin C

INTRODUCTION

Acute kidney injury (AKI) is a serious, potentially life-threatening condition which has been occurring with increasing frequency among hospitalized patients,[1;2] and is responsible for substantial healthcare-related costs.[3] In critically ill patients, cardiac surgery is the second most common cause of AKI after sepsis,[4] with approximately 1-5% of patients requiring dialysis following surgery. Post- cardiectomy AKI is associated with high morbidity and mortality,[5] prolongs hospital stay and results in increased costs.[6;7] Risk factors for the development of AKI after cardiac surgery include time on cardiopulmonary bypass (CPB),[8;9] older age, mitral valve surgery, combined valve surgery, and coronary arterial bypass grafting.[10] The mechanisms leading to AKI after cardiac surgery are multifactorial. Hemodynamic factors such as non-pulsatile flow during CPB decreasing perfusion of the renal cortex, low cardiac output, systemic vasodilatation and disturbed adrenergic and renin-angiotensin system activation, as well as microembolisation and nephrotoxic drugs may contribute to AKI. Another possible pathway to AKI is an immunological response, due to complement, neutrophil and monocyte activation, on the renovascular endothelium leading to impaired kidney function.[11-13]

Serum creatinine has traditionally been used for the assessment of kidney function after cardiac surgery, based on the assumption that its production and excretion are in balance. In clinical practice an abrupt rise in serum creatinine has been regarded as a sign of AKI, under the assumption that an acute decrease in glomerular filtration rate (GFR) results in the proportional accumulation of waste products in the blood.[14] In spite of its widespread use, serum creatinine remains a flawed diagnostic method for monitoring of GFR, and consequently for the diagnosis of AKI. Among other shortcomings, serum creatinine level is a function not only of excretion

through glomerular filtration, but also production mainly by muscle cells and secretion via tubular cells. Because of this, conditions that affect muscle mass and tubular secretion (such as age, gender, race and level of GFR) may influence the baseline level and the degree of rise in serum creatinine.[15] Another condition specific to cardiac surgery is the hemodilution induced with CPB, which results in a decrease in serum creatinine concentration[16] and could make a creatinine-based diagnosis of AKI difficult. Finally, a rise in serum creatinine occurs relatively late in the course of AKI,[17] which makes early diagnosis and intervention difficult when relied upon as the main diagnostic tool.

Recently, serum cystatin C has been shown to be a reliable marker of kidney function after cardiac surgery.[18-20] Cystatin C is a 13,25 kDa protein produced by all nucleated cells in the human body.[21] It is freely filtered in the glomerulus, then reabsorbed by epithelial cells in the proximal tubule where it is metabolized and not returned to the systemic circulation.[22] Serum cystatin C holds certain advantages over serum creatinine, in that it is not affected by the demographic characteristics influencing serum creatinine level, and its levels are also unaffected by conditions causing muscle injury.[21] Nevertheless, hemodilution should affect serum cystatin C levels equally, and its value in the early diagnosis of AKI remains controversial. In some studies serum cystatin C appeared to diagnose changes in kidney function more rapidly,[23] but other studies failed to confirm this.[24;25]

The performance of both serum creatinine and cystatin C in diagnosing AKI after CPB could thus be affected by processes that impact their serum concentration independent of changes in GFR. It is unclear which of the two biomarkers is affected less by such processes, and hence which one of them represents a less biased marker of GFR. We examined the temporal

dynamic of the changes in serum creatinine and cystatin C in patients with normal pre-operative kidney function undergoing CPB.

SUBJECTS AND METHODS

The Regional Medical Ethics Committee at Linköping University, Sweden approved the study according to the Helsinki declaration. Written informed consent was obtained from each study participant.

Patient population

We included 19 patients who underwent coronary artery bypass grafting in combination with valve procedures, surgery including two or more valves or in combination with other cardiac/ascendic aorta procedures between November 2010 - March 2011 at the Department of Cardiothoracic and Vascular Surgery, Linköping University Hospital, Linköping, Sweden. Patients with pre-existing chronic kidney disease (defined as an estimated GFR <60 ml/min/1.73m²), those participating in other research studies, and patients requiring emergency surgery were excluded.

Patients underwent anesthesia and monitoring according to the routine procedures of the department, and as required by the type of surgery. CPB was used in non-pulsatile mode with a mean arterial blood pressure (MAP) of 60 mmHg $\pm$ 10 mmHg. Mild hypothermia of about 35°C was used. Systemic vascular resistance and consequently MAP were maintained with phenylephrine or noradrenaline.

Blood samples and assays

Parallel samples of both serum creatinine and serum cystatin C were obtained during the first 72 hours after completion of CPB. Blood samples obtained on the day of hospital admission were used as baseline reference values. Blood samples were then obtained as specified in the

research protocol immediately post-CPB from the patient's arterial line, and postoperatively as shown in **Figure 1**. Baseline and immediate post-CPB hematocrit were obtained concomitantly with serum creatinine and cystatin C in all patients to determine the degree of hemodilution. Serum creatinine values obtained throughout the patients' hospitalization after the first 72 hours post-CPB were obtained from their medical chart, and were used to determine the presence or absence of AKI defined based on the Acute Kidney Injury Network (AKIN) criteria.[26] Serum creatinine and cystatin C were measured at the local clinical chemistry laboratory (SWEDAC accredited Lab Med in Östergötland, Sweden) using standard assays. The modified Jaffe method, using rate-blank measurement and intercept correction was used for assaying of serum creatinine using the Advia 1800 analyzer (Siemens Healthcare Diagnostics). For the assaying of serum cystatin C, the Advia 1650/1800 analyzer was used, based on determination of proteins by an immunochemistry technique and turbidimetric measurement with DakoCytomation reagents (Siemens Healthcare Diagnostics). Coefficients of variation for the analyses were: Cystatin C level 1: up to 1.14 mg/l CV=4.1%; level 2: up to 4.3mg/l, CV=3.9%. Creatinine level 1: up to 90 μ mol/l CV=4.3%; level 2 up to 380 μ mol/l CV=3.1%.

Statistical analyses

All results are presented as means $\pm$ standard deviations, or as median with interquartile range if appropriate. Variables were tested for fitting normal distribution. Based on an assumption of a normally distributed difference between matched pairs of biomarkers with standard deviation of 8 for serum creatinine and 0.15 for cystatin C[27] we estimated that a sample of 13 patients is needed to detect a 10% difference between matched pairs with a power of 0.9 and a type I error probability of 0.05. Within-patient differences for serum creatinine and serum cystatin C were compared by repeated measures ANOVA; in order to account for multiple

comparisons, differences between the levels of either biomarker at individual time points compared to their baseline values were examined by Tukey's HSD test. Sensitivity analyses were performed by excluding 2 patients who subsequently developed AKI.

Differences were considered statistical significant if $p < 0.05$. All statistical analyses were performed with STATISTICA version 10 (StatSoft, Inc., Tulsa, OK, USA) and Stata version 12 (Stata Corp., College Station, TX, USA).

RESULTS

Baseline clinical characteristics of the 19 patients are shown in **Table 1**. Patients had a mean (SD) age of 69.6 (9.3) years, 37% were females, and only one patient had diabetes mellitus. Blood transfusion was administered in 6 out of the 19 patients; all patients were in positive volume balance and none of the patients experienced oliguria. The median length of hospitalization was 5 days (25-75 percentile: 3-8 days), and two patients developed AKI based on the AKIN criteria for serum creatinine.[26]

Baseline serum levels of creatinine and cystatin C correlated significantly ($r=0.67$, $p=0.0015$, **Figure 2**). Overall both serum creatinine and cystatin C levels changed significantly during the entire length of follow-up ($p<0.001$ for both in repeated measures ANOVA), but the changes were not linear. Both serum creatinine (mean $\pm$ SD decrease from baseline of $16\pm 10\%$) and serum cystatin C (mean $\pm$ SD decrease from baseline of $10\pm 10\%$) decreased immediately following CPB (**Figures 3 and 4, and Supplemental Figures 1 and 2**). A concomitant, but more marked post-CPB decrease was seen in blood hematocrit level (mean $\pm$ SD decrease from baseline of $25\pm 8\%$). The initial decrease in creatinine and cystatin C was followed by an increase in their serum concentration, and a level equal to the baseline was observed approximately 12

hours following CPB. Serum creatinine levels measured at all time points after 12 hours post-CPB were not significantly different from its baseline level (**Figure 3 and Supplemental Figure 1**). In the case of cystatin C a further rise was observed following the 12-hour post-CPB time point, with subsequent levels being significantly higher compared to its baseline level at 48 and 72 hours (**Figure 4, and Supplemental Figure 2**). The results remained quantitatively and qualitatively similar when excluding the 2 patients who subsequently developed AKI (data not shown).

DISCUSSION

We examined temporal changes in serum levels of creatinine and cystatin C following cardiac surgery with CPB. We describe similar patterns for the two solutes in the first 24 hours post-CPB, consistent with a hemodilution effect. However, the post-CPB decrease in the serum concentrations of creatinine and even more so for cystatin C appeared to be less than what was expected based on the concomitant decrease in blood hematocrit value (a marker of hemodilution), indicating that processes causing a concomitant rise in their serum concentration (such as increased production or decreased excretion) might have blunted the effect of hemodilution. During the 24 to 72 hour post-CPB period serum creatinine levels stabilized, but serum cystatin C displayed an increase above its baseline level.

Serum creatinine has been the traditional serum biomarker used to estimate GFR and to diagnose AKI in clinical practice.[14] In spite of its many shortcomings (such as the influence of demographic characteristics and body composition on its serum concentration, and its inability to signal AKI immediately upon its occurrence[15;17]) and the emergence of various alternative biomarkers for GFR estimation and for AKI diagnosis, serum creatinine still remains the primary

diagnostic tool towards these ends. Monitoring of GFR for the accurate and timely diagnosis of AKI in cardiac surgery is of particular importance, given its common occurrence in this setting and its significant negative consequences.[4-7] Besides its usual shortcomings (vide supra), serum creatinine levels are also affected by unique circumstances during and after CPB. The hemodilution that occurs during and after CPB decreases the concentration of blood solutes,[16] and makes a timely diagnosis of AKI even more challenging.

Serum cystatin C is a biomarker that has emerged as an alternative to serum creatinine for estimating GFR.[18-20;28;29] It holds advantages over serum creatinine in that it is unaffected by the demographic characteristics that determine muscle mass and hence serum creatinine level, and its serum levels are also unaffected by tubular secretion.[21;22] Cystatin C has been shown to be a reliable marker of GFR in patients undergoing cardiac surgery,[18-20] but its ability to supersede serum creatinine for monitoring changes in GFR in the immediate post-operative period (which is the most important time period for the development of AKI) remains controversial. Some studies have suggested that serum cystatin C may be able to diagnose post-operative AKI more rapidly than serum creatinine,[23] but others have been unable to confirm this.[24;25] Hemodilution after CPB should affect serum levels of creatinine and cystatin C equally,[16] and while serum cystatin C levels are immune to many of the factors affecting serum creatinine levels, there are other conditions that impact serum cystatin C levels independent of GFR (such as obesity, diabetes mellitus, inflammation, very high doses of glucocorticoids and thyroid dysfunction[22;23;30]), raising theoretical questions about the ability of serum cystatin C to serve as an unbiased marker of GFR. Indeed, our results suggest that serum creatinine and cystatin C display significantly different changes in the post-CPB period, with serum creatinine stabilizing at levels equal to baseline, but serum cystatin C rising further to

significantly higher levels. One explanation for this phenomenon could be that serum cystatin C is a more sensitive marker of GFR and hence it was able to diagnose a decrease in GFR (i.e. AKI), while serum creatinine did not. An alternative explanation, though, is that serum cystatin C levels were raised via increased production through some mechanism induced by cardiac surgery and CPB (e.g. acute inflammation[31]), hence leading to a false-positive diagnosis of AKI. It is thus possible that the faster rise in serum cystatin C compared to serum creatinine during AKI in some studies[23] is due to the fact that the level of the former is also affected by ancillary processes that occur in parallel with the acute decline in GFR, such as inflammation. Future studies will have to address this question by including direct measurement of GFR and/or novel biomarkers of AKI.[32;33]

The results of the present study have to be interpreted with the recognition of its limitations. We examined a limited number of patients with normal kidney function from a single institution, hence the external validity of our results will have to be confirmed by other studies. We did not include in our assessment direct measures of GFR, and we did not test for the presence of AKI through means such as markers of tubular damage,[34;35] which makes it difficult to determine the exact reasons for the observed differences in the levels of serum creatinine and cystatin C. While none of our patients experienced AKI during the evaluation period according to accepted diagnostic criteria (urine output and/or a significant rise in serum creatinine or cystatin C), novel biomarkers such as serum or urine neutrophil gelatinase associated lipocalin (NGAL) have been shown to detect subclinical AKI,[36] hence its use could have been beneficial. We also did not test novel biomarkers such as symmetrical dimethyl arginine (SDMA), which has been suggested to be a better measure of GFR than serum creatinine-based equations in patients with normal kidney function or with AKI.[37]

CONCLUSIONS

We describe significant differences in the changes of serum levels of creatinine and cystatin C following CPB, suggesting that the two biomarkers may not be equally well suited to monitor GFR in this setting. Such differences may affect not only the comparative effectiveness of serum creatinine and cystatin C as diagnostic markers of AKI, but also their performance as prognostic indicators of clinical outcomes. It remains to be determined in future studies if the discrepant post-CPB concentration dynamics of serum creatinine and cystatin C are related to differences in their production rates, differences in their ability to detect small changes in GFR, or to a combination of these.

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TRANSPARENCY DECLARATIONS

None of the authors declared any conflict of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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Table 1. Baseline clinical characteristics of the 19 patients studied.

Age (years)		69.6±9.3 (51-86)
Gender (M/F N, %)		12 (63)/7 (37)
Body surface area (m ²)		1.96±0.22 (1.52-2.36)
Diabetes mellitus (N, %)		1 (5)
Total blood loss (ml)		-686±456 (-1,800-0.0)
Blood transfusion during CPB (ml)		750±528 (300-1,800)
Hematocrit pre-CPB		0.43±0.049 (0.34-0.51)
Hematocrit post-CPB		0.32±0.041 (0.26-0.41)
Urine output during CPB (ml)		550±223 (230-1,000)
Urine output 24 hours post-CPB (ml)		3,261±629 (2,105-4,395)
Total fluid balance (ml)		5,425±1,296 (3,550-8,650)
Time to extubation (minutes)		404.4±299.5 (165-1,105)
CBP time (minutes)		157.1±49.2 (80.0-283.0)
Maximum Mean Arterial Pressure (mmHg)		70.4±4.7 (65.0-80.0)
Minimum Mean Arterial Pressure (mmHg)		52.6±4.8 (45.0-65.0)
Medications	Norepinephrine (µg/kg/min, N=9)	0.048±0.058 (0.0-0.2)
	Furosemide (mg, N=2)	15.0±7.07 (10.0-20.0)
	Mannitol (g, N=19)	59.1±9.6 (45.0-75.0)

Data are presented as means ± standard deviation (range), or number (%).

M: male. F: female. CPB: cardiopulmonary bypass.

Figure legend

Figure 1

Schematic overview of the blood sampling time intervals for serum creatinine and serum cystatin

C. The shaded black area represents the time under cardiopulmonary bypass.

Figure 2

Correlation between serum cystatin C and serum creatinine at baseline (n=19) and at 72 hours post CPB (n=15). R represents the Spearman correlation coefficient.

Figure 3

Mean (SD) percent change from baseline in serum creatinine values plotted over the first 72 hours postoperatively. N=19; *p<0.05 compared to baseline.

Figure 4

Mean (SD) percent change from baseline in serum cystatin C values plotted over the first 72 hours postoperatively. N=19; *p<0.05 compared to baseline.

Supplemental Figure legend

Online Figure 1

Mean (SD) serum creatinine values plotted over the first 72 hours postoperatively. N=19;

*p<0.05 compared to baseline.

Online Figure 2

Mean (SD) serum cystatin C values plotted over the first 72 hours postoperatively. N=19;

*p<0.05 compared to baseline.