Evaluation of serum cysteine-rich protein 61 and cystatin C levels for assessment of acute kidney injury after cardiac surgery

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ABSTRACT

Objective The occurrence of acute kidney injury (AKI) after cardiopulmonary bypass (CPB) can lead to morbidity and mortality. We hypothesized that cysteine-rich protein 61 (CYR61) and cystatin C (CysC) may be potential novel biomarkers of AKI after cardiopulmonary bypass.

Methods Patients were classified into AKI and non-AKI group depending on serum creatinine. Levels of creatinine, CysC, and CYR61 were measured at five time-points before and within 48 h after the surgery.

Results Fifty patients were included in the study. Serum creatinine pre-operative values were 74.0 \( \pm \) 43.3 l mol/L in AKI group vs. 64.8 \( \pm \) 17.9 l mol/L in non-AKI group. During 48 h, the values increased to 124.6 \( \pm \) 67.2 l mol/L in AKI group (\( p \) < 0.001) but in non-AKI group they did not change significantly. Serum CysC values were significantly increased already 2 h after CPB in AKI group (949 \( \pm \) 557 l g/L, \( p \) < 0.05) compared to non-AKI group (700 \( \pm \) 170 l g/L). Pre-operative serum CYR61 tended to be lower in AKI group (12.4 l g/L) than in non-AKI group (20.3 l g/L), but 24 h after the surgery, the levels in AKI group tended to be higher than non-AKI group.

Conclusion Serum CYR61 does not seem to be an early predictor of AKI in patients after cardiac surgery with CPB, but it might possibly identify patients at risk of developing more severe kidney injury. Serum CysC could be a promising biomarker of AKI, differentiating patients at risk of developing AKI after cardiac surgery as early as 2 h after surgery.

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Introduction

Acute kidney injury (AKI) is a serious progressive problem that affects patients after cardiac surgery and increases the rate of morbidity and mortality considerably. The incidence of AKI among hospital-acquired patients ranges from 5% in patients with normal kidney function up to 25% in ICU patients. The etiology of AKI has three major origins: pre-renal that might occur due to hypovolemia, renal hypoperfusion, coagulopathies, congestive heart failure, and cirrhosis; renal which resulted from glomerulonephritis, acute tubular necrosis, interstitial nephritis, and vasculopathies; and post-renal which can be developed from renal calculi, urinary tract obstruction, bladder malignancy, prostatic hyperplasia and blood clots. Therefore, early screening and diagnosis of AKI is an important step in preventing and optimizing therapy. The classification of AKI is dependent on KDIGO criteria, a new updated harmonized RIFLE (Risk, Injury, Failure, Loss, and ESRD) criteria and AKIN (Acute Kidney Injury Network) criteria and relies on serum creatinine within 48 h and urinary output if available.

The traditional AKI marker is creatinine which remains unchanged until 50% of kidney function falls down. It is affected by non-specific factors like diet, age, dehydration, muscle mass, gender, and drugs; and these influences can result in poorer diagnosis and wasted time of treatment decisions. Cystatin C (CysC), a cystatin protease inhibitor, is less affected by non-specific factors. When GFR decreases by the action of catabolism in proximal renal tubules, CysC begins to increase. CysC was recommended by several authors to be measured in addition to creatinine in GFR estimation.

Recent studies suggested that the concentration of CysC 24 h after surgery as well as pre-operative CysC values were associated with AKI. Cysteine-rich protein (CYR61) is an extracellular matrix molecule consisting of four distinct domains. The secretion of CYR61 is relevant for different physiological functions including angiogenesis, fibrosis, migration,
proliferation, differentiation, development, apoptosis, and senescence development. CYR61 is massively abundant at sites of inflammation, wound healing, it is accompanied with chronic inflammation and tissue injury. Furthermore, Quan et al. discovered that overexpression of CYR61 levels were possibly due to sensitivity to environmental stress, and/or hypoxic conditions. Studies on mice after unilateral kidney ischemia reperfusion injury (IRI) suggested that blocking CYR61 could reduce the inflammatory consequences associated with ischemic AKI. The data on serum CYR61 in AKI is scarce; therefore, the process of working on serum CYR61 may introduce more information about AKI which occurs after cardiac surgery with cardiopulmonary bypass (CPB).

This study aimed to evaluate whether the serum levels of CysC and CYR61 could be used as early biomarkers for the identification of patients who are prone to AKI before and after cardiac surgery with CPB.

**Patients, materials, and methods**

**Patients**

Our study included patients who were admitted to Department of Cardiovascular Surgery at the University Medical Center Ljubljana for elective cardiac surgery with CPB. The study was carried out according to the declaration of Helsinki. The National Medical Ethics Committee of the Republic of Slovenia approved the study protocol and an informed consent was obtained from all study participants prior to data collection. The inclusion criteria were ages from 20 to 80 years and normal kidney function as assessed by creatinine. The exclusion criteria were patients with diabetic nephropathy, lupus nephritis, overlapping syndromes, malignancies, pregnancy, and hemodialysis or kidney transplantation. The patients were classified into AKI group and non-AKI group according to KDIGO criteria dependent on serum creatinine levels, data for all patients were summarized in Table 1. In AKI group, there were 18 patients with AKI stage 1 and 8 patients with AKI stage 2.

**Materials and methods**

Blood samples were collected from all patients at five time-points which are: before surgery, 2, 24, and 48 h after the end of CPB. Thus, blood samples were collected in tubes without additives. After collection, samples were centrifuged to obtain serum. Serum aliquots were stored at −20°C until analysis. Subsequently, serum creatinine was measured using automated assay based on modified kinetic Jaffe reaction (Siemens Healthcare Diagnostics Inc., Newark, DE) with limit of detection 9 μmol/L. For the measurement of serum CysC and CYR61 ELISA kits were used (Bio Vendor GmbH, Heidelberg, Germany and Elabscience Ltd, Wuhan, China, respectively). The limit of detection was 0.2 μg/L for CysC and 2 μg/L for CYR61. Moreover, Estimated GFR (eGFR) was calculated using Modification of Diet in Renal Disease (MDRD) formula.

The results were reported as mean ± standard deviation (SD) or median and interquartile ranges for cases without normal distribution. Statistical analysis was performed by a software package SPSS version 22 (SPSS Inc., Chicago, IL); p values <0.05 was considered to be significant. For creatinine and CysC, means were compared using Student t-test and ANOVA test with post-hoc Bonferroni analysis. Medians and interquartile ranges were obtained by non-parametric Mann–Whitney U test for CYR61. The performance of biomarkers was assessed by area under the curve (AUC) values.

**Results**

Fifty patients were classified into AKI group and non-AKI group according to KDIGO criteria dependent on serum creatinine values, data for all patients were summarized in Table 1. In AKI group, there were 18 patients with AKI stage 1 and 8 patients with AKI stage 2.

Serum creatinine levels were significantly raised from the baseline in AKI group 2 h after CPB (93.3 ± 38.7 μmol/L) with p < 0.01 and reached the high peak plateau after 48 h of CPB in AKI group (124.6 ± 67.2 μmol/L) with p < 0.001. In non-AKI group, serum creatinine levels were significantly decreased from the baseline immediately after CPB (62.0 ± 16.4 μmol/L) with p < 0.01. Thereafter, there was a transient increase within 24 h (68.1 ± 20.8 μmol/L) with p = 0.001, which dropped back to the pre-operative

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**Table 1. A summary of clinical data of the patients.**

|                      | Non-AKI | AKI     | p    |
|----------------------|---------|---------|------|
| Number of patients and gender (male/female) | 24 (12/12) | 26 (18/8) | 0.409 |
| Age (years)          | 79.8 ± 10.9 | 70.1 ± 13.4 | 0.770 |
| BMI (kg/m²)          | 27.2 ± 4.4 | 27.3 ± 4.7 | 0.841 |
| Diabetes mellitus (non/oral/insulin) | 20/2/2 | 15/7/4 | 0.448 |
| Arterial hypertension (y/n) | 18/6 | 24/2 | 0.520 |
| Left ventricular ejection fraction (%) | 59.7 ± 16.1 | 59.2 ± 7.3 | 0.718 |
| Pre-operative eGFR (mL/min/1.73 m²) | 95.1 ± 22.1 | 93.7 ± 34.1 | 0.531 |
| eGFR ≤ 60 mL/min/1.73 m² (number of patients) | 1 | 6 | – |
| CPB time (min)       | 97 ± 33 | 101 ± 37 | 0.053 |

Data are represented as mean ± SD; BMI: Body Mass Index; eGFR: Estimated Glomerular Filtration Rate; CPB: Cardiopulmonary bypass.
Data are represented as mean ± SD. Significance at $p < 0.05$. Pre: before surgery; 0 h: at the end of CPB; 2, 24, and 48 h: hours after the end of CPB.

| Measured parameters          | Non-AKI ($N = 24$) | AKI ($N = 26$) | $p$  |
|-----------------------------|---------------------|----------------|------|
| Creatinine pre (µmol/L)     | 64.8 ± 17.9         | 74.0 ± 43.3    | <0.001|
| Creatinine 0 h (µmol/L)     | 62.0 ± 16.4         | 83.3 ± 28.1    | <0.001|
| Creatinine 2 h (µmol/L)     | 69.7 ± 14.3         | 93.3 ± 38.7    | <0.001|
| Creatinine 24 h (µmol/L)    | 68.1 ± 20.8         | 111.9 ± 55.6   | <0.001|
| Creatinine 48 h (µmol/L)    | 64.6 ± 22.4         | 124.6 ± 67.2   | <0.001|

Figure 1. Serum creatinine values (mean and SD) at five time-points: before surgery, at the end of CPB and 2, 24, and 48 h after the end of CPB.

Serum CysC levels in AKI group gradually increased from the initial baseline to 2 h after CPB (949 ± 557 µg/L) with $p < 0.05$. They continued to increase to reach 1.5-fold the baseline within 48 h (1421 ± 739 µg/L) with $p < 0.01$, exhibiting similar dynamics to serum creatinine. However, a slight decline in serum CysC levels in non-AKI group was noticed 2 h after CPB (700 ± 170 µg/L) with $p < 0.05$ but then it persisted to increase up to (910 ± 422 µg/L) with $p < 0.01$ within 48 h as shown in Table 3. In addition, the serum CysC concentrations at different time points in AKI and non-AKI groups are presented in Figure 2.

As CYR61 values were not normally distributed, medians and interquartiles were used instead of mean ± SD. The median baseline of serum CYR61 was higher in non-AKI group (20.3 µg/L) than in AKI group (12.4 µg/L) but the difference was not significant. Consequently, both groups experienced sudden drops in median values immediately after CPB and regained the pre-operative levels within 2 h (Figure 3). After 48 h, the median values were higher than the baseline in AKI group but in the non-AKI group they reached not more than half the value of the baseline. A comparison of serum CYR61 median values between AKI and non-AKI groups did not show any significant difference as depicted in Table 4. However, 2 h after CPB, the comparison of groups with AKI stages 1 and 2 gave a lower $p$ value, which was closer to significance ($p = 0.066$). Furthermore, a significant negative correlation was observed between serum CYR61 48 h after the end of CPB and age of the patients ($p < 0.01, r = -0.465$) as represented in Figure 4. A receiver operating characteristic (ROC) curve analysis of the creatinine, CysC, and CYR61 values within 2 h after CPB gained the areas under the curve 0.767, 0.655, and 0.513, respectively, as shown in Figure 5.

Discussion

Our findings revealed that serum creatinine levels increased more ($p < 0.001$) within 48 h post-operatively in AKI group than non-AKI group. However, the pre-operative serum creatinine baseline was higher in AKI group than non-AKI group. This difference might be explained by reduced pre-operative renal function with elevated state of inflammation due to endothelial dysfunction. These results are compatible with the study of Ishani et al., who mentioned that serum creatinine levels
were increased in all patients after cardiac surgery, exceeding the pre-operative baseline. Conversely, Sirota et al. showed that serum creatinine level was significantly decreased pre-operatively in AKI group in comparison to non-AKI group; and was overexpressed post-operatively in AKI group than non-AKI. In contrast to adult population, a study which was carried out on pediatric cardiac patients by Peco-Antić et al. stated that no significant change was observed in serum creatinine baseline between AKI and non-AKI groups.

Rosner and Moran et al. explained the increase in CysC levels in AKI after CPB as a consequence of renal tubular damage which affected upstream glomerular filtration due to congestion. Accordingly, Zheng et al. noticed that post-operative serum concentrations of CysC in AKI group initially declined for 6 h, then subsequently rose again in 12 h, and got stabilized between 24 and 48 h after CPB. In our study, serum CysC levels were decreased 2 h after CPB in non-AKI group only. In AKI group, we observed early significant increase of CysC levels.

Although CysC was recommended by some authors to be measured in addition to creatinine in GFR estimation, it has not found its place in early detection of AKI after cardiac surgery. Creatinine, which had the highest AUC in our study, still seems to be the favorable single choice for early detection of AKI despite its limitations. A test panel including creatinine and new markers could add some value with improved specificity and fewer influences of all the factors that can affect creatinine results. Another challenge for the use of new markers remains the cost-effectiveness, but this important question exceeds the scope of our study.

Serum CYR61 baseline levels were found to be lower in AKI group than in non-AKI group. Although the difference was not significant, it is interesting due to the reversed ratio between AKI and non-AKI group on the first day after surgery, when serum CYR61 levels were higher than baseline in AKI group and lower than baseline in non-AKI group. The ratio was similar on the second post-operative day. This pattern is not yet well understood and the role of CYR61 in AKI needs to be further clarified.

We also noticed that serum CYR61 levels 2 h after CPB were different between the subgroups with AKI stage 2 and milder forms of AKI like AKI stage 1 (p = 0.066). The difference was not significant due to small number of patients in subgroups, but nevertheless the observation seems intriguing.

These findings suggest that serum CYR61 levels failed to discriminate AKI early after cardiac surgery with CPB. However, CYR61 might possibly identify patients with more severe kidney injury, which would be very beneficial for early treatment of AKI after cardiac surgery. With the results of our pilot study we cannot confirm this possible role of CYR61; further research involving larger groups of patients is needed.

Muramatsu et al. found that urinary CYR61 started to appear at 3–6 h, reached its maximum peak concentration at 6–9 h after kidney injury, and disappeared after volume depletion. Volume depletion was accompanied with cardiac surgery by the effect of pump machine. Hence, this might also explain the drop of serum CYR61 level during CPB in our study. One of the strengths of our study is measuring CYR61 in serum samples, which usually give more reliable results than urine samples and also represent the true level of the analyte in the circulation at one given moment. To the best of our knowledge, no research on serum CYR61 in AKI after surgery using human samples has been published.

The broad inter-individual variations of CYR61 concentrations were reported by Hviid et al., who observed that CYR61 levels increased at sites of tissue injury like surgical wound closure which was characterized by

### Table 4. Serum CYR61 levels (μg/L) in AKI and non-AKI groups at different points.

| Measured parameters | Non-AKI (N = 24) | AKI (N = 26) | p     |
|---------------------|-----------------|-------------|-------|
| CYR61 pre (μg/L)    | 20.3            | 12.4        | 0.47  |
| Q1–Q3               | 7.9–51.7        | 3.7–42.8    |       |
| CYR61 0 h (μg/L)    | 5.7             | 4.0         | 0.82  |
| Q1–Q3               | 1.8–19.8        | 2.4–13.9    |       |
| CYR61 2 h (μg/L)    | 12.4            | 8.9         | 0.88  |
| Q1–Q3               | 4.8–24.9        | 6.0–45.2    |       |
| CYR61 24 h (μg/L)   | 10.4            | 14.2        | 0.39  |
| Q1–Q3               | 1.2–24.6        | 4.2–28.2    |       |
| CYR61 48 h (μg/L)   | 11.8            | 15.9        | 0.62  |
| Q1–Q3               | 4.0–29.9        | 4.0–54.9    |       |

CYR61: Cysteine-rich protein 61. Data are represented as mean ± SD. Significance at p ≤ 0.05. Pre: before surgery; 0 h: at the end of CPB; 2, 24, and 48 h: hours after the end of CPB.
inflammatory activation. Great variability of CYR61 levels observed in our study could be at least partly influenced by similar factors and response to renal hypoperfusion without intensive ischemic acute kidney injury. However, in some studies, CYR61 was activated by cytokine exposure and promoter region of NF-κB binding-sites without de novo protein biosynthesis.

One of the limitations of our study is that some influences on the occurrence of AKI like serum lactate were not addressed. Although the causal relationship was not

![Figure 4](image-url)  
**Figure 4.** The correlation ($r = -0.465; p < 0.01$) between serum CYR61 levels (μg/L) 48 h after the end of CPB and age of the patients (years).

![Figure 5](image-url)  
**Figure 5.** ROC curve for serum creatinine, CysC and CYR61 values 2 h after the end of CPB.
established, lactate was found to be independently associated with AKI. These potential influences could be addressed in the future studies.

Conclusion

Although serum CYR61 does not seem to be an early predictor of AKI in patients after cardiac surgery with CPB, it might possibly identify patients at risk of developing more severe kidney injury, which would be very beneficial for early treatment. This possible role of CYR61 still needs further research involving larger groups of patients.

Serum CysC was found to differentiate patients at risk of developing AKI as early as two hours after cardiac surgery. The role of CysC as a marker of kidney function had been recognized by many studies, so this marker could be an added value in early evaluation of AKI after cardiac surgery using CPB, especially in combination with other markers.

Disclosure statement

All authors declare that they have no conflicts of interest.

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