

# Long-Term Consequences of Increased Activity of Urine Enzymes After Cardiac Surgery – A Prospective Observational Study

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**Introduction:** Cardiac surgery associated AKI (CSA-AKI) complicates recovery and may be associated with a greater risk of developing chronic kidney disease and mortality. The aim of this study was to assess long-term clinical consequences of transient increased activity of urinary enzymes after cardiac surgery (CS).

**Methods:** An observational study was conducted in a group of 88 adult patients undergoing planned coronary artery bypass grafting (CABG), but all samples were obtained from 79 patients. The activity of urinary enzymes: N-acetyl-beta-glucosaminidase (NAG), arylsulfatase A (ASA) and beta-glucuronidase was evaluated in sequential urine samples. A comparative analysis of biochemical parameters was performed regarding the occurrence of acute kidney injury (AKI) defined by KIDGO at 24 hours, at day 30 and 5-years after the operation.

**Results:** During the first 24 hours after CS AKI was diagnosed in 13 patients. A comparison of the activity of urinary enzymes in pre-defined time-points showed significant differences for ASA and NAG (post OP-sample  $p < 0.028$  and  $p < 0.022$ ; POD 1 sample  $p < 0.004$  and  $p < 0.001$  respectively). No patient had any biochemical or clinical features of kidney failure at day 30. In the AKI group kidney failure was diagnosed in 36% of patients within 5 years of follow-up as opposed to 5% in the no AKI group. The activities of tubular enzymes in urine reflect a general injury of kidney tubules during and after the operation. However, they are not ideal biomarkers for prediction of the degree of kidney injury and further poor prognosis of CS-AKI.

**Keywords:** acute kidney injury, AKI, N-acetyl-beta-glucosaminidase, NAG, arylsulfatase A, ASA, beta-glucuronidase, B-GP, cardiac surgery

## Introduction

Acute kidney injury (AKI) is an important clinical problem. Cardiac surgery associated AKI (CSA-AKI) complicates recovery from cardiac surgery in up to 30% of patients and may be associated with 50–60% mortality.<sup>1,2</sup> The patients who survive CSA-AKI have a greater risk of developing chronic kidney disease or end stage renal disease compared to patients without AKI and about 2% to 5% of them patients require renal replacement therapy.<sup>3,4</sup>

During cardiac surgery performed with cardio-pulmonary bypass (CPB) the time of kidney injury is predictable and usually occurs secondary to the unique characteristics of cardiac procedures. The pathomechanism of AKI is complex and caused by an interplay between patient susceptibility to injury and many additional factors. The dominant and potentially reversible factors are those related to ischemia and reperfusion injury.<sup>5</sup>

The gold standard of AKI diagnosis, based on the KDIGO (Kidney Disease Improving Global Outcomes) definition, which includes only serum creatinine concentration and urinary output changes, is insufficient in many clinical situations

and may lead to delay in diagnosis and treatment.<sup>6</sup> These traditional laboratory approaches for detection of CSA-AKI may react relatively late to the consequences of renal tubular injury.<sup>5</sup> Serum creatinine may be insensitive to detect mild kidney injury since the kidney can maintain glomerular filtration even if many nephrons are damaged.<sup>7</sup> Therefore, the concept of “subclinical AKI” has been developed, with elevated biomarkers but normal serum creatinine. Some authors have found that it has been linked to impaired prognosis.<sup>5,8</sup> Therefore, there is a need for a new definition of AKI not based on a change in serum creatinine but mainly based on temporal change of the level of multiple biomarkers and clinical parameters that can be detected early after renal injury. Because all injurious factors lead to the kidney damage, what affects mainly the tubules, early monitoring of proximal tubule function seems to be a promising new tool to predict kidney dysfunction after cardiac surgery.

Recent work by Nejat et al indicated that even in cases of “transient” AKI, high sensitivity markers of tubular damage can be present, showing probable staging of an evolving spectrum of AKI.<sup>9</sup> Urinary markers have been proven to be more sensitive for true histological damage. An elevation of serum biomarkers, like creatinine, are probably more sensitive for changes in clearance.<sup>7</sup>

N-acetyl-beta-glucosaminidase (NAG) is a lysosomal brush border enzyme present in proximal renal tubules, but also widely distributed. NAG cannot be filtered by the glomerulus in the kidney, because of the high molecular weight (140 kDa). NAG is normally secreted in small concentrations because of physiological exocytosis process, but its excretion rises as a result of acute lesions. Lysosomal arylsulfatase A (ASA) is an enzyme required to degrade sulfatides, which were found in the kidneys. Changes in sulfatide levels may be correlated with kidney dysfunction but have not been studied in cardiac surgery population regarding AKI. The results of other studies suggest that arylsulfatase A has a protective effect on kidney allograft function.<sup>10</sup> Beta-glucuronidase (B-GU) is a physiologically important lysosomal glycosyl hydrolase widely distributed in mammalian tissues, body fluids and microbiota.<sup>11</sup> Urine beta-glucuronidase (B-GU) activity has not been studied in the cardiac surgery population.

The normal values for urine enzymes activity in a healthy population or after cardiac surgery (after cardiopulmonary bypass as intervention) are unknown, but there are a few publications discussing this problem. Up to date the researchers have studied the diagnostic utility of increased activity of urine enzymes separately and in combination for the detection of AKI after cardiac surgery.<sup>12–15</sup>

A question arises whether perioperative determination of the activity of urinary enzymes could serve as a good marker for the diagnosis of CSA-AKI in practice? Or, rather, is it a marker of transient renal tubular insufficiency without further consequences? Currently, there is no definitive answer to this question. Therefore, any additional information helpful in the understanding of reasons for postoperative kidney failure would enable the establishment of proper diagnostic and therapeutic process. Therefore, the aim of this study was to evaluate the long-term clinical consequences of increased activity of urinary enzymes measured within the first 24 hours after cardiac surgery as markers of CSA-AKI.

## Materials and Methods

### Study Population

A prospective observational study was conducted in a group of consecutively enrolled 88 adult Caucasian patients (above the age of 18 years), all samples were obtained from 79 patients. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethical Committee of the Pomeranian Medical University in Szczecin (Poland) (KB – 0012/146/10). The protocol of the study was registered prospectively at the ClinicalTrials.gov database (identifier NCT03860545).

An informed consent form was obtained from all patients who underwent an elective procedure of cardiac artery bypass grafting with the use of cardio-pulmonary bypass at the Department of Cardiac Surgery of the Pomeranian Medical University in Szczecin (Poland) and agreed to participate in this project. According to the study protocol the following exclusion criteria regarding the pre-operative period were defined:

- re-operations,
- a known pathology of the urinary tract, renal failure,

- chronic use of the following medications: non-steroidal anti-inflammatory drugs (NSAIDs), immunosuppression, steroids in the preoperative period,
- active autoimmune diseases,
- active neoplastic diseases,
- active local or generalized infection.

## Study Definitions

Early Acute Kidney Injury (AKI) was defined according to the AKIN criteria achieved within the first 24-hours after surgery.<sup>6</sup> The damage of proximal renal tubules was diagnosed in pre-defined time points by increased activity of brush border enzymes: N-acetyl-beta-glucosaminidase (NAG), Lysosomal arylsulfatase A (ASA), beta-glucuronidase (B-GR).

No features of kidney injury after 30 days post operation was recognized as “transient changes”.

## Study Protocol

Before the cardiac procedure, clinical assessment and a full physical examination was performed to assess patient's clinical status and perioperative risk and to qualify them for the study. In the operating theatre, standard patient monitoring was introduced. An induction of general anesthesia was performed using drugs doses calculated per body weight: fentanyl (load: 3–5 mcg/kg, maintenance: 0.5–1 mcg/kg), etomidate (load: 0.2 mcg/kg) and pancuronium (load: 0.04–0.1mg/kg; maintenance 0.01mg/kg), and sevoflurane was used for maintenance of anesthesia using up to 1–1.3 minimal alveolar concentration (MAC). Standard parameters were monitored continuously: ECG, invasive blood pressure monitoring, central venous pressure, deep body temperature (rectal) and mechanical ventilation. Fluid balance and diuresis were evaluated on hourly basis during the duration of surgery and for the first 24 hours postoperatively. Additional monitoring was performed with a blood gas analyzer: hemoglobin, hematocrit and electrolyte analysis in arterial blood using the GEM 3000 machine (USA). The surgery was performed in normothermia, which was achieved by heating mattress and heated fluids. Cardiopulmonary bypass was performed using a non-pulsatile pump (Maquet, Hirrlingen, Germany) and membrane oxygenator (Terumo, California, USA). We used priming with 500 mL of Gelofusine, 1000 mL of Ringer's lactate, 60 mEq of sodium bicarbonate and 4 mg/kg of heparin. An individual CPB flow was calculated based on 2.5 L/min/m<sup>2</sup> rule and systemic arterial blood pressure was maintained between 50 and 70 mmHg. If systemic perfusion pressure decreased, an intervention was introduced: CPB flow was increased up to a maximum of 130% of calculated flow and/or we titrated intravenous norepinephrine infusion.

## Biochemical Evaluation

Serum creatinine level and other standard laboratory parameters (full blood count, biochemistry) were evaluated on the day of the operation and 24 hours post-operatively. The NGAL concentration in the urine was measured using a commercially available ELISA kit (Human Lipocalin –2/NGAL Quantikine ELISA Kit, R&D Systems R&D System, Minneapolis, MN, USA) with microplate reader ELx808 (BIO-TEK Instruments, Inc., USA), according to the manufacturer's instructions. The time points for NGAL measurement were as follows: 1 hour after the CPB (post-OP sample) and 24 hours from the beginning of the operation (postoperative day 1, POD sample).

The activity of N-acetyl-beta-glucosaminidase (NAG), beta-glucuronidase (B-GR) and arylsulfatase A (ASA) were measured in single urine samples. The method of biochemical studies was as follows: after collection the urine the samples were centrifuged at 4000 rpm for 10 min. Urine without the sediment was stored at –80°C until the time of analysis. The activity of NAG was measured by the Maruhn colorimetric method using p-nitrophenyl-N-acetyl- $\beta$ -D glucosamine (Sigma-Aldrich) as a substrate (Maruhn 1976). The p-nitrophenol, which was released in the reaction, was evaluated by measuring the sample compared to the blank sample absorbance at a wavelength of  $\lambda=405$  nm.

The arylsulfatase A activity was determined according to the Baum colorimetric method modified by Werner. This assay was based on using p-nitrocatechol sulfate dipotassium salt (Sigma-Aldrich) as a substrate.<sup>16,17</sup> The amount of p-nitrocatechol liberated in the reaction was established by measuring the absorbance of samples at 515 nm in alkaline solution.

The activity of beta-glucuronidase was measured by the Szasz colorimetric method using p-nitrophenyl-beta-D-glucuronide (Sigma-Aldrich) as a substrate.<sup>18</sup> The p-nitrophenol, which was freed in the reaction, was determined by measuring the absorbance of samples at a wavelength of  $\lambda=405$  nm. Moreover, the concentration of creatinine in the urine was assessed in a reaction with picric acid, after dilution of the urine by 50 times.

The time points for measurement of urine enzymes activity were preoperative (baseline = zero sample), 1 hour after the CPB (post-OP sample) and over 24 hours from the beginning of the operation (postoperative day 1, POD sample).

After diagnosis of early AKI was made, the study population was divided into two groups:

- Group I “no AKI” (n = 66),
- Group II “AKI” (n = 13).

## Outcomes and Follow-Up

We analyzed the changes of serum creatinine concentration and enzymuria in relation to the occurrence of acute kidney injury according to AKIN criteria up to 24 hours post-op.

Next, we analyzed the following medical scenarios: the need for CRRT, clinical observation (general status of the patient, hemodynamic stability and need for mechanical ventilation support), and complications: features of kidney injury in the 30 days after the cardiac procedure and 30-day mortality. Finally, we analyzed 5-year all-cause mortality and history of kidney failure. The data were collected from a questionnaire, by phone or during the visit in the outpatient clinic.

The flow diagram of the study is depicted in [Figure 1](#).

## Statistical Analysis

The statistical analysis was performed using STATISTICA 10 software (StatSoft Poland). The normality of the data distribution was verified using the Shapiro–Wilk test. The results are presented as mean  $\pm$  standard deviation (SD). To determine the differences between the AKI group vs non-AKI group the U Mann–Whitney rank test was used for quantitative variables and Pearson’s Chi-square test was used for qualitative variables. The Wilcoxon rank sum test was used to compare urinary biomarker levels at each time point between patients with and without AKI. Statistical significance was set at  $p < 0.05$ .

## Results

After diagnosis of early AKI was made, the study population was divided into two groups:

- Group I “no AKI” (n = 66), age  $60 \pm 7$  years, range 45–81 years, 55 men
- Group II “AKI” (n = 13), age  $70 \pm 6$  years, range 61–79 years, 9 men.

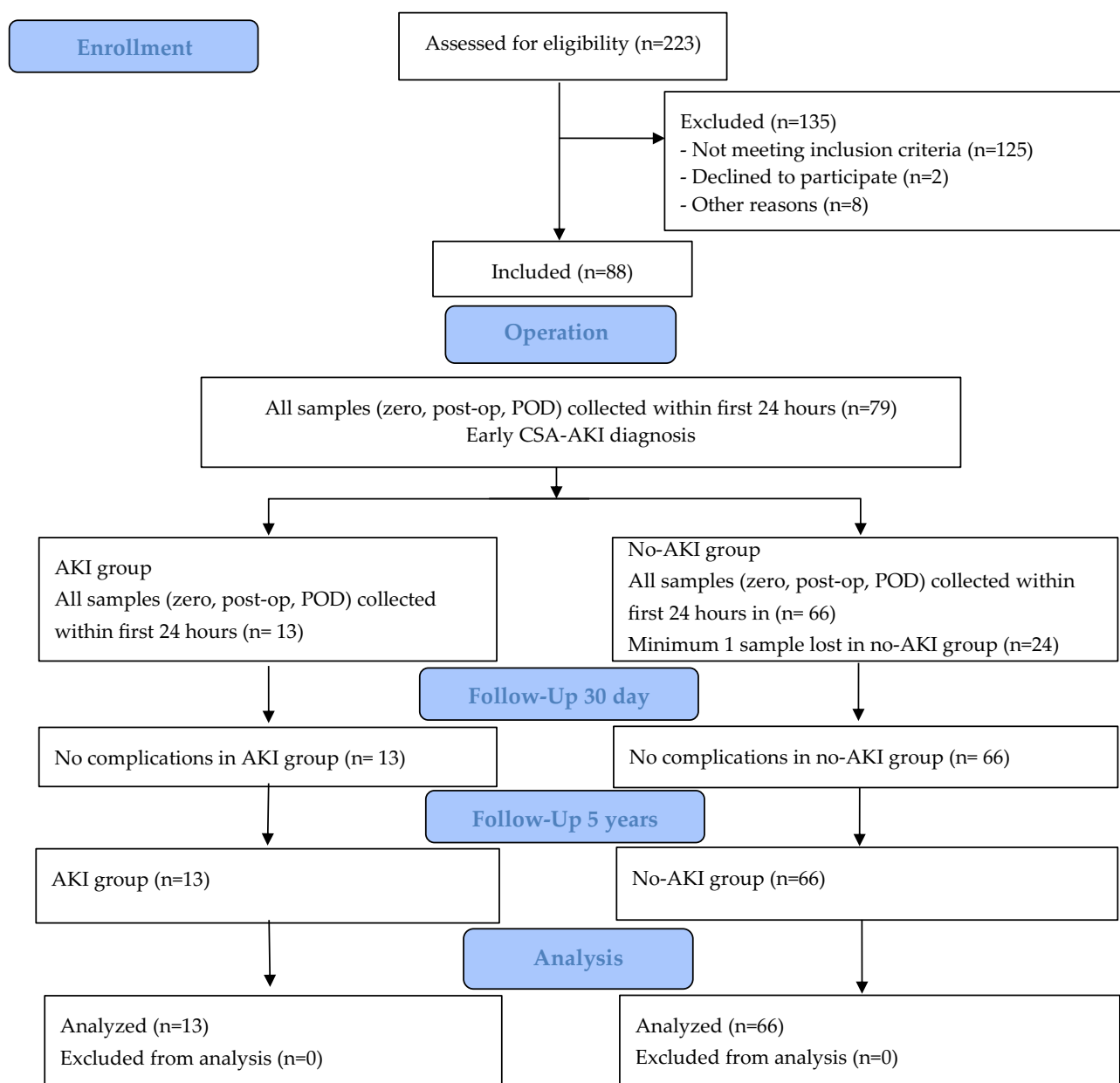
During the first 24 hours after cardiac surgery AKI was diagnosed in 13 patients (16%), in 6 cases AKIN stage 1 (next progression to stage 2 based on oliguria) and in 7 patients’ AKIN stage 2 was diagnosed ([Figure 1](#)). In all cases, the AKIN diuresis criteria were equivalent to the AKIN creatinine criteria. The characteristics of the patient population are shown in [Table 1](#).

When comparing majority of the parameters: the duration time of the cardiopulmonary bypass, aorta cross-clamping time, surgical procedure time, the minimal value of the mean arterial pressure (MAP), the need for vasopressors or inotropes, diuresis and fluid balance and minimal body temperature during CABG, there were no differences between the subgroups in the intraoperative period. Mean CPB time in both groups was  $48 \pm 15$  min. Mean aorta cross-clamping time was  $29 \pm 7$  min in both groups. The diuresis was significant lower in the AKI group during the postoperative period ( $p = 0.007$ ). None of the patients needed perioperative transfusion of blood products. The kinetics of each enzyme are presented in [Figure 2A–C](#).

A comparison of serum creatinine, NAG, ASA, B-GP activity and NGAL excretion in the urine in pre-defined time-points are shown in [Table 2](#).

No patient had any biochemical or clinical features of kidney failure at day 30 after cardiac surgery.

During the 5-years follow-up 2 patients (15%) died, and 4 patients (36%) suffered from chronic kidney failure in “AKI group”. Within the “no-AKI group” 7 patients (10%) died and 3 patients (5%) developed chronic kidney failure.



**Figure 1** Flow diagram.

**Abbreviations:** AKI, acute kidney injury; CSA-AKI, cardiac surgery associated acute kidney injury; POD, post-operative day.

The cause of kidney failure was not finally established. None of the patients needed dialysis. The kinetics of each enzyme regarding long term follow up are present in [Table 3](#).

## Discussion

The main finding of the present study is the confirmation of the existence of transient damage of proximal renal tubules diagnosed by increased activity of brush border enzymes early after cardiac surgery using cardiopulmonary bypass. However, the question remains open, whether it is just an indicator of tubular damage or an early AKI predictor.

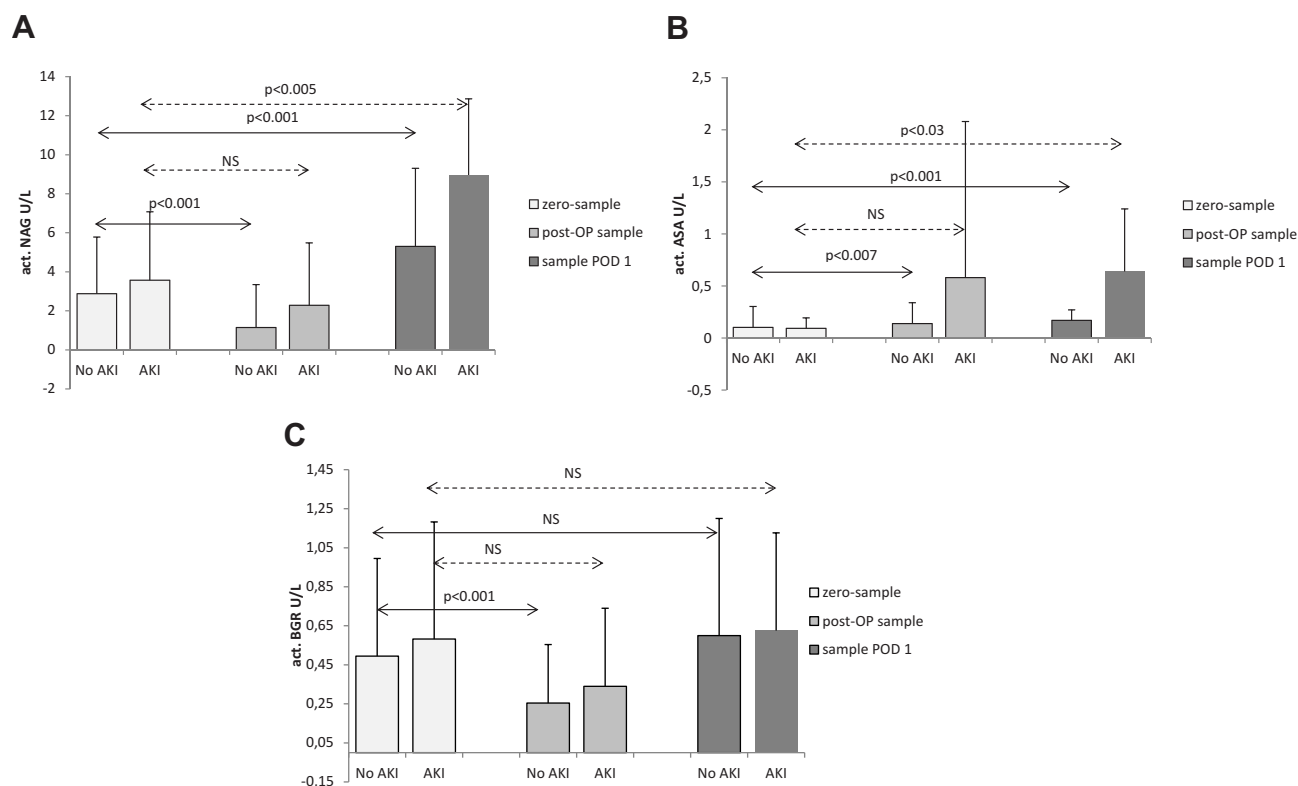
In the present study changes of the biomarkers over time indicate the presence of proximal tubular injury after procedures using cardiopulmonary bypass. Both, the kinetics, and peak levels of absolute values of NAG and ASA activity were significantly higher in AKI group as compared to the no-AKI group within the first 24-hours after surgery. We did not observe a similar result for B-GR activity. No features of kidney injury were noticed beyond 30 days after

**Table I** Demographic Data and Co-Morbidities of the Study Population

Characteristics	No AKI N = 66	AKI N = 13	p value
Age (years) (mean $\pm$ SD)	60 $\pm$ 6	70 $\pm$ 6	<0.001
BMI (mean $\pm$ SD)	28 $\pm$ 3	31 $\pm$ 3	0.017
LV EF before the surgery (mean $\pm$ SD)	53 $\pm$ 9	46 $\pm$ 12	0.098
Gender (male) (n, %)	55 (83)	9 (69)	0.236
Left ventricular dysfunction NYHA $\geq$ II (n, %)	41 (62)	11 (83)	0.140
Myocardial infarction (n, %)	29 (44)	10 (77)	0.029
Arterial hypertension (n, %)	56 (84)	11 (84)	0.450
Diabetes mellitus (n, %)	19 (28)	8 (61)	0.022
Peripheral vascular disease (n, %)	55 (83)	10 (77)	0.716
Atrial fibrillation (n, %)	2 (3)	1 (13)	0.660

**Abbreviations:** AKI, acute kidney injury; BMI, body mass index; LV EF, left ventricle ejection fraction before procedure (%); NYHA, New York Heart Association; SD, standard deviation.

surgery. Regarding the classic CSA-AKI we called this phenomenon “transient changes”. However, in the AKI group kidney failure was diagnosed in 36% of patients within 5 years of follow-up as opposed to 5% in the no AKI group. This finding indicates the necessity of paying increased attention to patients presenting with “transient changes” for a longer time after cardiac surgery procedure.


**Figure 2** The comparison of NAG (A), ASA (B) and B-GR (C) activity in pre-defined time-points among the AKI group and no-AKI group.

**Abbreviations:** AKI, acute kidney injury; ASA, lysosomal arylsulfatase A; B-GR, beta-glucuronidase; NAG, N-acetyl-beta-glucosaminidase; POD, post-operative day.

**Table 2** Peri-Operative Values of Urine and Serum Parameters in the Study Population

Characteristics (Mean $\pm$ SD)	No AKI N = 66	AKI N = 13	p value
<b>Pre-operative (zero sample)</b>			
NAG U/L (urine)	2.884 $\pm$ 2.9	3.575 $\pm$ 3.5	0.500
ASA U/L (urine)	0.103 $\pm$ 0.2	0.093 $\pm$ 0.1	0.382
B-GR U/L (urine)	0.495 $\pm$ 0.5	0.582 $\pm$ 0.6	0.781
Creatinine mg. dl <sup>-1</sup> (serum)	0.876 $\pm$ 0.1	0.95 $\pm$ 0.27	0.416
<b>1 hour after the end of CPB (post-OP sample)</b>			
NAG U/L (urine)	1.146 $\pm$ 2.2	2.281 $\pm$ 3.2	0.022
ASA U/L (urine)	0.139 $\pm$ 0.2	0.580 $\pm$ 1.5	0.028
B-GR U/L (urine)	0.254 $\pm$ 0.3	0.340 $\pm$ 0.4	0.596
NGAL ng.mL <sup>-1</sup> (urine)	3.093 $\pm$ 4.6	12.558 $\pm$ 22.1	0.020
<b>24 hours after the beginning of the operation (sample POD 1)</b>			
NAG U/L (urine)	5.305 $\pm$ 4.0	8.962 $\pm$ 3.9	0.004
ASA U/L (urine)	0.170 $\pm$ 0.1	0.640 $\pm$ 0.6	<0.001
B-GR U/L (urine)	0.600 $\pm$ 0.6	0.626 $\pm$ 0.5	0.596
Creatinine mg.dl <sup>-1</sup> (serum)	0.851 $\pm$ 0.1	1.28 $\pm$ 0.1	<0.001
NGAL ng.mL <sup>-1</sup> (urine)	12.489 $\pm$ 12.1	25.057 $\pm$ 11.8	<0.001

**Abbreviations:** AKI, acute kidney injury; ASA, lysosomal arylsulfatase A; B-GR, beta-glucuronidase; NAG, N-acetyl-beta-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; CPB, cardio-pulmonary bypass; POD, post-operative day; SD, standard deviation.

**Table 3** The Kinetics of Each Enzyme Regarding Long Term Follow Up

5-year Follow Up (Mean $\pm$ SD)	AKI n=4	AKI n=2	AKI n=7	No-AKI n=7	No-AKI n=3
<b>Death</b>	No	Yes	No	Yes	No
<b>ChRF</b>	Yes	No	No	No	Yes
<b>NAG [U/g]*</b>	0.97 $\pm$ 0.6	1.02	2.90 $\pm$ 2.23	1.83 $\pm$ 2.21	3.18 $\pm$ 2.69
<b>NAG [U/g]**</b>	22.99 $\pm$ 17.52	8.82	10.77 $\pm$ 8.26	8.76 $\pm$ 7.86	10.8 $\pm$ 10.31
<b>NAG [U/g]***</b>	9.79 $\pm$ 3.02	3.26	4.82 $\pm$ 2.95	3.57 $\pm$ 2.09	3.31 $\pm$ 2.97
<b>ASA [U/g]*</b>	0.01 $\pm$ 0.02	0.06	0.06 $\pm$ 0.06	0.03 $\pm$ 0.05	0.28 $\pm$ 0.43
<b>ASA [U/g]**</b>	1.89 $\pm$ 0.73	1.63	2.16 $\pm$ 2.69	6.82 $\pm$ 13.95	2.93 $\pm$ 2.54
<b>ASA [U/g]***</b>	0.70 $\pm$ 0.78	0.19	0.33 $\pm$ 0.29	0.17 $\pm$ 0.16	0.12 $\pm$ 0.12
<b>BGR [U/g]*</b>	0.09 $\pm$ 0.09	0.14	0.63 $\pm$ 0.78	0.38 $\pm$ 0.37	0.23 $\pm$ 0.17
<b>BGR [U/g]**</b>	2.56 $\pm$ 1.76	2.69	2.77 $\pm$ 3.42	5.98 $\pm$ 11.86	1.73 $\pm$ 1.34
<b>BGR [U/g]***</b>	0.48 $\pm$ 0.31	0.21	0.41 $\pm$ 0.42	0.35 $\pm$ 0.23	0.21 $\pm$ 0.28
<b>NGAL**</b>	32.40 $\pm$ 34.34	1.60	4.35 $\pm$ 2.94	3.95 $\pm$ 2.75	1.70 $\pm$ 1.23
<b>NGAL***</b>	30.95 $\pm$ 11.17	22.07	22.54 $\pm$ 9.55	11.51 $\pm$ 9.6	7.31 $\pm$ 3.4
<b>Creatinine*</b>	1.09 $\pm$ 0.44	0.82	0.92 $\pm$ 0.18	0.86 $\pm$ 0.16	0.92 $\pm$ 0.17
<b>Creatinine*</b>	1.46 $\pm$ 0.46	0.87	1.31 $\pm$ 0.32	0.93 $\pm$ 0.17	0.84 $\pm$ 0.13

**Notes:** \*Zero sample, \*\*1 hour after the end of CPB (post-OP sample), \*\*\*POD – post-operative day, “5 year follow up” - 5 years after cardiac surgery.  
**Abbreviations:** AKI, acute kidney injury; ASA, lysosomal arylsulfatase A; B-GR, beta-glucuronidase; NAG, N-acetyl-beta-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; ChRF, chronic renal failure.



Our study was designed to answer the question: what are the long-term consequences of the increased activity of urine enzymes early after cardiac surgery? The results of our study show that the answer is ambiguous. Up to date, the problem of “transient” damages of proximal renal tubules with or without further AKI is discussed.<sup>12</sup> The true histological postoperative damage would be confirmed by a kidney biopsy, but this management seems to be too harmful. The clinical scenarios after injury caused by cardiac surgery signalize that transient histological lesion can relate to functional impairment can maintain glomerular filtration despite nephrons being damaged. The mechanisms responsible for triggering an inflammatory process leading to tubular atrophy and interstitial fibrosis are different in individual cases. It may explain why some patients with perioperative AKI may or may not develop chronic renal failure.

The temporal expression patterns of urinary biomarkers before the development of postoperative AKI were also studied. It has been demonstrated in a small group of patients that a panel of urinary biomarkers may improve early detection of tubular damage and postoperative AKI diagnosis.<sup>5</sup> However, some studies have shown that the urinary NAG activity increases also in patients who do not develop clinical AKI after surgery.<sup>14,15</sup>

Up to date, the definition for AKI is still based on a change in the level of serum creatinine. Because of limited data of validation and availability, the biomarkers are not used in routine clinical practice today.

The mechanism of ischemia-reperfusion injury was explored in different clinical situations after kidney transplantation. The conclusion of a study presented by Kwiatkowska et al was that the increased excretion of tubular enzymes occurred even in the absence of morphological evidence of tubular damage.<sup>10</sup> The authors emphasized that lysosomal activity of tubular cells rises when protein filtration is higher. This mechanism might explain the correlation between urinary NAG concentration and severity of proteinuria. The authors analyzed the outcome of renal transplant patients and noticed that NAG level evaluated in the early postoperative period could act as a prognostic marker of a long-term allograft function. In this specific group of patients tubular enzymuria is not only an indicator of tubular damage, but also a predictor of the long-term function of transplanted kidney and renal allograft rejection.<sup>10</sup>

The search for a perfect biomarker for CSA-AKI is still ongoing. For this purpose, single biomarkers or their panels (including NAG, NGAL) and changes in their concentration over time were examined.<sup>7,10</sup> When we consider the clinical utility of new biomarkers of AKI, we expect: 1) diagnostic value (used to identify a disease), 2) prognostic value (used to determine the progression in patients who have the disease), 3) predictive value (used to identify cases who are more likely than similar individuals without the elevated marker to experience a disease from exposure for risk factors). Statistic methods to assess objectively above problems are the optimal combination of sensitivity and specificity, AUROCs, PPV and NPV. However, this information cannot be depicted in many studies. There is a variety of results from declared negative to very optimistic. It may generate some statistical pitfalls when translating results to clinical practice. Few papers referred to as the state of the art on the diagnostic usefulness of biomarkers in AKI, including papers reporting results in adult cardiac surgery.

Results reported on the use of biomarkers in cardiac surgery are disappointing and conflicting. Available literature provides twenty-seven different definitions for AKI, which further complicates drawing of any conclusions. The number of patients in the studies varied from 30 to 1219 and the number of events varied from 1 to 85.<sup>12–14,18–21</sup> The AUROCs of urinary enzymes varied from 0.27 to 0.98. Positive Predictive Values and Negative Predictive Values ranged from 4% to 100% and from 61% to 100%, respectively. An explanation for this wide range of results leads to a conclusion that it is associated with the heterogeneity of AKI definitions, the number of analyzed cases, different types of cardiac surgery, mixed population of adults and children, different points of observation (intraoperatively, 30 minutes after CPB till 24 hours after CPB), the assessment of biomarkers for early prediction of need for renal replacement therapy, the underlying conditions causing AKI (eg, sepsis, critically ill patient), and co-morbidity (diabetic nephropathy, hypertension, chronic kidney disease). That may explain, why the results of current studies are difficult to compare and impossible to implement a common cut-off point and introduction to clinical practice. Moreover, there is a need to redefine the term of early cardiac surgery associated AKI, which should not be based on the change in serum creatinine level and urine output but preferably based on a panel of multiple biomarkers that are able to detect early renal injury within minutes to hours. Maybe this “transient pathology”, now called “subclinical” AKI, will become an important problem in the context of long-term observation.



One of the studies discussing the topic compared a panel of early biomarkers of AKI (NAG, NGAL) for the detection of AKI in patients undergoing heart surgery. The authors observed that NAG rose and reached a peak value within 4 hours after admission to the ICU, likely reflecting the acute tubular ischemia and injury following surgery, and then quickly returning to normal. Urinary NAG was elevated 4 hours after injury in patients with AKI as compared to those without AKI and decreased soon after to baseline. The authors explained that the reasons for this rapid normalization may reflect the relatively benign nature of kidney injury.<sup>13</sup> Ho et al performed an analysis on the release of a tubular injury marker, evaluated every 30 minutes during CPB, in addition to post-CPB and post-operative sampling in the ICU to detect early biomarker signs of tubular injury. The authors found that NAG doubled, compared to baseline, already 30 min after the initiation of CPB and remained elevated throughout the CPB period. The authors have proven that only the CPB duration was an independent predictor of tubular injury.<sup>19</sup> Quite contrary, the results of our study do not confirm this thesis.

Liangos et al performed a comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass.<sup>22</sup> The purpose of their study was to compare the performance of six candidate urinary biomarkers, measured 2 hours following CPB for the early detection of AKI in a prospective cohort of 103 patients undergoing cardiac surgery. AKI developed in 13% of the patients. Only urinary KIM-1 remained independently associated with AKI after adjustment for a preoperative AKI prediction score or CPB perfusion time.

In clinical practice, we try to define what degree of subclinical damage, as detected by increased activity of urinary enzymes, will be important and linked with a poor long-term outcome. The lack of evidence of outcome needs future exploration. The aim of the Lanemyr et al. study was to analyze the release of NAG during and early after CPB and to describe independent predictors of maximal tubular injury.<sup>5</sup> In this study of the kinetics of a biomarker indicative of proximal tubular injury during and after cardio-pulmonary bypass, the marker was found to rise early after the initiation of bypass and to return to normal levels on the day after surgery. Peak levels were not predictive of the development of acute kidney injury. In our study we had similar observations. Some authors found that the rise in urinary NAG may increase also in patients who do not develop clinical AKI after surgery. It is comparable to our results.

The clinical meaning of increased activity of urine enzymes during the postoperative period remains uncertain in the patients without renal complications after operation. The reverse of increased activity of urine enzymes could be explained that the patients are carefully monitored and treated during the first few days after cardiac procedures.<sup>1,3,4</sup> Up to date, there is no validated cut-off point for discrimination of clinically significant tubular damage during intraoperative or early postoperative periods in adults. While AUROC values look impressive, applying levels of urinary or serum biomarkers for discrimination in individual patients is disappointing by a wide overlap between groups and false positive results as consequences. Despite the increasing availability of rapid point-of-care tests for many renal biomarkers, the current evidence does not support their routine clinical use for early prediction of AKI after cardiac surgery in adults. It may explain why we still must use the old suboptimal AKI definitions based on serum creatinine and urine output.<sup>20</sup> However, early diagnosis of CS-AKI is of critical importance to prevent any associated complications. According to Hayiroğlu et al. AKI is an independent prognostic factor for long-term mortality among patients with ST-segment elevation myocardial infarction complicated by cardiogenic shock and treated with primary percutaneous coronary intervention (PPCI).<sup>21</sup> Moreover, Çinar et al demonstrated the predictive value of age, creatinine, ejection fraction score for in-hospital mortality in patients with cardiogenic shock.<sup>22</sup> Therefore, future approach using machine learning-assisted monitoring combining both clinical information and specific biomarkers is a novel and promising approach with better prediction of AKI after cardiac surgery or in patients suffering from cardiogenic shock than traditional, commonly used models.<sup>23–29</sup>

Our study is not without limitations. Although prospective, it was a single center study; therefore, a prospective sample with a larger volume of patients to confirm our observations would be very important. Moreover, due to organizational issues we included a relatively small number of patients, therefore this effect should also be taken into consideration. Finally, the presence of some confounding preoperative factors which can be considered as having some influence on renal tubular function must be accounted for in this study. Nevertheless, the authors strongly believe that

information collected within this study is of paramount importance to the experts in the field, especially in the context of long-term follow-up.

## Conclusion

Our results support the previous findings that biomarkers reflect a general lesion of kidney tubular injury during and after cardiac procedures in adults. NAG, ASA, B-GR urine activity are not ideal biomarkers for prediction of the degree of kidney injury and further poor prognosis. Even “transient changes” regarding former early CSA-AKI were diagnosed and our finding indicates necessity of increased care of these patients for a long time after procedure.

## Data Sharing Statement

De-identified participant data will be available to other researchers only upon a reasonable written request. Please contact the corresponding author, e-mail: katarzyna.kotfis@pum.edu.pl.

## Disclosure

The authors report no conflicts of interest in this work.

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