Urinary netrin-1 predicts early ischemic acute kidney injury after cardiopulmonary bypass

Mohammad H. Aly[a], Alsayed Alnahal[a], Goerge Emad[a], Abdel A.M. Jumaa[b], Mohammad M. El Shaarawy[c]

Departments of [a]Internal Medicine, [b]Medical Biochemistry, [c]Cardiothoracic Surgery, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Correspondence to Alsayed Alnahal, MBBch, MSc, MD, Department of Internal Medicine, Zagazig University Hospital, PO Box 44519, Zagazig, Sharkia, Egypt.
e-mail: alnahal2002@yahoo.com

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Background
Serum creatinine is an unreliable early biomarker for the diagnosis of acute kidney injury (AKI) after cardiac surgery. We need to search for a rapid and dependable marker for the detection of AKI.
Aim of work
This study was designed to test urinary netrin-1 (Ntn1) as a marker of early kidney injury post cardiac surgery. Our study included 39 patients with preoperative normal creatinine. We measured serum creatinine and urinary Ntn1 at 0, 6, and 24 h after cardiac surgery, AKI was defined according to the KDIGO 2012 definition.
Results
Fourteen patients developed AKI after cardiac surgery. There was statistically significant elevation in urinary Ntn1 at 6 and 24 h after cardiopulmonary bypass (CPB) surgery in the AKI group, while serum creatinine failed to show any statistically significant elevation at 6 h after CPB in the same group. No statistically significant change was seen in the level of creatinine or urinary Ntn1 at 6 and 24 h after CPB surgery in the non-AKI group.
The sensitivity and specificity of urinary Ntn1 to detect AKI at 6 h after CPB surgery was 86.7 and 91.7%, respectively, at a cutoff value of 107.3 pg/ml. Combined urinary Ntn1 and serum creatinine had the same sensitivity and specificity.
Conclusion
Urinary Ntn1 may be considered as an early sensitive biomarker of AKI at 6 h after cadiopulmonary bypass surgery instead of serum creatinine that rises only 24 h after CPB surgery in patients with cardiac surgery-associated acute kidney injury.

Keywords:
acute kidney injury, cardiopulmonary bypass, netrin-1

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Introduction

Acute kidney injury (AKI) is a common and serious postoperative complication of cardiac surgery requiring cardiopulmonary bypass (CPB), and the second common cause of AKI in the ICU [1].

Bothc oronary artery bypass grafting and surgery for valvular disease are most common modules of surgical procedures [2]. The incidence of AKI is increasing worldwide, affecting about 6% of all hospitalized patients [3].

AKI is an abrupt deterioration in kidney function following cardiac surgery as evidenced by a decrease in the glomerular filtration rate; detection of AKI may be delayed in the first 24–48 h depending on the serum creatinine levels. Thus, the need for a more reliable, earlier indicators and predictors of AKI has emerged. The causes of AKI postcardiac surgery may be toxins, metabolic abnormalities, ischemia, reperfusion injury, neurohormonal activation, inflammation, and oxidative stress [4]. Many studies have reported that even mild increases in serum creatinine levels following cardiac surgery were associated with significant effects on mortality [5], long-term survival was depending AKI duration [6], and early recovery of renal function was associated with improved long-term survival after cardiac surgery-associated acute kidney injury (CSA-AKI) [7].

Mortality rate in CSA-AKI in higher in patients requiring renal replacement therapy than in patients not requiring renal replacement therapy [8].

The netrins are laminin-related proteins, which were discovered as a kidney injury marker during spatial and

temporal expression studies in the kidney after ischemia followed by reperfusion [9].

Within hours after reperfusion, netrin-1 (Ntn1) protein expression appears in the proximal tubular epithelial cells; it also appears in the urine and can be quantified immediately after reperfusion, so Ntn1 was discovered as an early diagnostic biomarker of kidney injury [10].

Aim of the work

To study the value of urinary Ntn1 as an early biomarker of ischemic AKI in CPB patients in our hospital.

Patients and methods

This work has been carried out in Zagazig University Hospitals, during the period from December 2013 to November 2014.

Patients

All patients who underwent cardiac surgery using CPB at the Cardiothoracic Surgery Department of Zagazig University Hospitals were included in the study except those who were excluded by the exclusion criteria; then the total patients were divided into two main groups (AKI and non-AKI groups) according to their postoperative rise of serum creatinine depending on the KDIGO definition of AKI as an increase in serum creatinine by more than 0.3 mg/dl within 48 h [11].

Exclusion criteria

All patients were selected to be free from the following:

Chronic kidney disease (KDIGO definition).
Hypertension(BP>140/90 mmHg).
Diabetes mellitus.
Liver diseases.
Collagen diseases.
Sepsis.
Malignancy.

All participants of the study were subjected to a written consent.

Methods

All participants were subjected to the following:

(1) Full medical history and complete clinical examination.
(2) Routine investigations:
    (a) Complete urinalysis.
    (b) Complete blood picture.
    (c) Fasting plasma glucose level
    (d) Liver function tests.
    (e) Serum creatinine, urea, and electrolytes such as sodium and potassium.
    (f) BMI was calculated by dividing weight (kg) by the square of height (m^2) [12].
(3) Specific investigation: included the following:
    (a) Measurement of urinary Ntn1 by enzyme-linked immunosorbent assay (ELISA) basal, 6 and 24 h after CPB surgery:
    Human Ntn1 ELISA kit used to assay Ntn1 in a sample of human serum, blood plasma, and other related tissue fluid.

Statistical analysis

Data were collected and analyzed using Statistical Package for the Social Sciences (version 20.0; SPSS Inc., Chicago, Illinois, USA). *P* value was set at less than 0.05 for significant results and less than 0.001 for highly significant results.

Results

During the period of the study, 39 patients who underwent cardiac surgery using CPB met the inclusion criteria and they were divided into two main groups:

Group A (AKI group)

In this group of patients, the serum creatinine was elevated either by 50% of the basal level or by absolute rise 0.3 mg/dl above the basal level 24 h after cardiac surgery. It included 15 patients (10 men and 5 women) with a mean age value of ±SD of 36.8± 8.6 years. They had a BMI with a mean value±SD of 26.3±3.2 kg/m^2.

Group B (non-AKI group)

It included 24 patients (13 women, 11 men) with a mean age value of 37.91±12 years. Basal serum creatinine was with mean value±SD of 0.78±0.16 mg/dl. CPB time (mean value±SD) was 44.3±11.7 min, ICU stay was 2.1±0.3 days. All patients of the study had average amount of urine output.

All results are demonstrated in detail in the following tables and figures:

Table 1 shows the clinical data of the studied groups (AKI and non-AKI); no statistically significant difference was found between the studied groups as regards age, BMI, systolic, diastolic blood pressure sex complete blood picture, fasting blood glucose, and prothrombin time, whereas a

statistically significant difference was found between both groups as regards the CPB time (min) and ICU stay (day).

No statistically significant difference was found between the studied groups as regards liver function, serum electrolytes, serum bicarbonate, basal serum creatinine, and basal urinary Ntn1 (pg/ml) (Table 2). But there was a high statistically significant difference found between the studied groups as regards urinary Ntn1 after 6 h (pg/ml), urinary Ntn1 after 24 h (pg/ml), and serum creatinine (24 h).

A statistically significant increase in mean value±S.D of both urinary Ntn1 (pg/ml) and serum creatinine (mg/dl) after 6 and 24 h, as compared with basal time in the AKI group, whereas no statistically significant difference was found along the time course for urinary Ntn1 and serum creatinine in non-AKI group as shown in Table 3.

Our study has shown a statistically significant positive correlation between (urinary Ntn1) 6 h after CPB versus CPB time (min) in the AKI group but no statistically significant correlation was detected in

Table 1 Comparison of some clinical and laboratory data between acute kidney injury and nonacute kidney injury groups

	AKI	Non-AKI	*t*-Test	*P*
Age (years)	36.8±8.6	37.91±12	0.472	0.618
BMI (kg/m^2)	26.3±3.2	25.5±2.9	1.258	0.457
Systolic BP (mmHg)	116.0±11.2	117.5±12.5	–0.377	0.708
Diastolic BP (mmHg)	74.0±6.32	73.3±7.01	0.299	0.766
CPB time (min)	60.6±10.9	44.3±11.7	2.229	0.041*
ICU stay (days)	4.6±1.3	2.1±0.3	8.360	<0.001**
Fasting blood glucose (mg/dl)	94.46±9.5	99.1±10.9	–1.356	0.183
Hb (g/dl)	12.9±1.3	13.2±1.3	–0.776	0.443
WBCs (×109/l)	6.06±1.5	6.68±1.3	–1.343	0.187
Plt (×10^9/l)	193.9±51.6	192.8±44.8	0.070	0.944
PT (s)	13.14±1.98	13.2±1.5	–0.228	0.821
Fasting blood glucose (mg/dl)	94.46±9.5	99.1±10.9	–1.356	0.183

AKI, acute kidney injury; CPB, cardiopulmonary bypass; Hb, hemoglobin; Plt, platelet; PT, prothrombin time; WBCs, white blood cells.
*Statistically significant. **Highly statistically significant.

Table 2 Comparison of some laboratory data between acute kidney injury and nonacute kidney injury groups

	AKI	Non-AKI	*t*-Test	*P*
AST (IU/l)	21.9±10.1	20.2±8.8	0.550	0.586
ALT (IU/l)	25.8±13.3	24.8±13.9	0.214	0.832
Total bilirubin (mg/dl)	0.72±0.3	0.75±0.32	–0.189	0.851
Direct bilirubin (mg/dl)	0.20±0.1	0.25±0.14	–0.940	0.354
Serum albumin (g/dl)	4.06±0.39	4.09±0.32	–0.301	0.765
Serum Na (mEq/l)	138.7±3.2	139.3±4.04	–0.519	0.607
Serum K (mEq/l)	4.21±0.6	3.96±0.34	1.626	0.112
Serum bicarbonate (mEq/l)	24.2±2.5	25±4.6	–0.607	0.547
Serum creatinine (basal) (mg/dl)	0.86±0.15	0.78±0.16	1.628	0.112
Urinary netrin-1 (basal) (pg/ml)	21.19±46.7	18.9±9.3	1.513	0.431
Serum creatinine after 6 h (mg/dl)	0.88±0.41	0.78±0.16	1.628	0.112
Serum creatinine after 24 h (mg/dl)	1.32±0.16	0.85±0.18	8.218	<0.001**
Urinary netrin-1 after 6 h (pg/ml)	255.17±82.3	54.8±45.6	8.560	<0.001**
Urinary netrin-1 after 24 h (pg/ml)	151.97±75.2	34.8±24.3	6.755	<0.001**

Table 3 Time course of the studied biomarker in patients with acute kidney injury versus those without acute kidney injury using repeated measures analysis of variance test (general linear model)

Patient category	Biomarker	Basal	6 h	24 h	*F*	*P*
Patient with AKI	Urinary netrin-1 (pg/ml)	21.19±46.7	255.17±82.3[a]	151.97±75.2[a,b]	27.6	<0.001**
	Serum creatinine (mg/dl)	0.86±0.15	0.88±0.41[a]	1.32±0.16[a,b]	14.07	<0.001**
Patient without AKI	Urinary netrin-1 (pg/ml)	18.9±9.3	24.8±15.6	21.8±24.3	0.68	0.50
	Serum creatinine (mg/dl)	0.78±0.16	0.82±0.18	0.85±0.18	0.98	0.37

[a]Statistically significant compared with the studied basal time biomarkers. [b]Statistically significant compared with the studied 6 h biomarkers.
**Highly significant.

the non-AKI group. There was no statistically significant correlation between urinary Ntn1 6 h after CPB (pg/ml) versus each of age, hemoglobin (g/dl), and serum creatinine 6 h after CPB (mg/dl) in both groups Table 4.

In Table 5, a statistically significant positive correlation can be seen between (urinary Ntn1 24 h after CPB versus CPB time (min) and serum creatinine 24 h after CPB in the AKI group, whereas no statistically significant correlation was detected as regards these parameters in the non-AKI group.

Both Table 6 and Fig. 1 show the validity of the studied biomarker (urinary Ntn1) as a predictor of AKI 6 h after CPB.

Setting a cutoff value of 107.3 (pg/ml) for urinary Ntn1 yielded a sensitivity and specificity of 86.7 and 91.7%, respectively, with a positive predictive value of 86.6% and an negative predictive value of 91.6%.

The combined sensitivity and specificity for urinary Ntn1 and serum creatinine was 86.7 and 91.7%, respectively, with a positive predictive value of 86.6% and an negative predictive value of 91.6%.

Discussion

Experimental studies had identified interventions that may prevent or treat AKI if started early in the disease process, before the serum creatinine rises [13]. The lack of early predictive biomarkers has impaired our ability to translate these promising findings to human AKI [14].

Our study showed statistically highly significant elevation in urinary Ntn1 at 6 and 24 h after CPB surgery in the AKI group, whereas there was no statistically significant elevation in serum creatinine at 6 h after CPB in the same group.

In the non-AKI group, both serum creatinine and urinary Ntn1 did not show any significant change at 6 or at 24 h after CPB compared with the baseline value.

This can be explained by the fact that within hours after reperfusion, Ntn1 protein expression appears in the proximal tubular epithelial cells but, at the same time, the expression in vascular endothelial cells is downregulated [14]. As Ntn1 is a secreted protein, it will be detected in the urine and quantified instantly after reperfusion [15].

Table 4 Correlation coefficient (*r*) value of urinary netrin-1 (pg/ml) after 6 h versus some studied parameters in acute kidney injury and nonacute kidney injury groups

Urinary Netrin-1 6 h after CPB (pg/ml) versus	AKI		Non-AKI	
	r	*P*	*r*	*P*
Age (years)	–0.43	0.11	0.47	0.12
Serum creatinine 6 h after CPB (mg/dl)	0.12	0.66	0.08	0.68
CPB time (min)	0.65	0.04*	–0.31	0.13
Hb (g/dl)	0.34	0.2	0.53	0.223

CPB, cardiopulmonary bypass; Hb, hemoglobin.

Table 5 Correlation coefficient (*r*) value of urinary netrin-1 (pg/ml) after 24 h versus some studied parameters in acute kidney injury and nonacute kidney injury groups

Urinary netrin-1 24 h after CPB (pg/ml) versus	AKI		Non-AKI	
	r	*P*	*r*	*P*
Age (years)	–0.16	0.55	0.35	0.08
Serum creatinine 24 h after CPB (mg/dl)	0.62	0.044*	0.16	0.44
CPB time (min)	0.33	0.05*	–0.36	0.18
Hb (g/dl)	–0.3	0.27	0.303	0.241

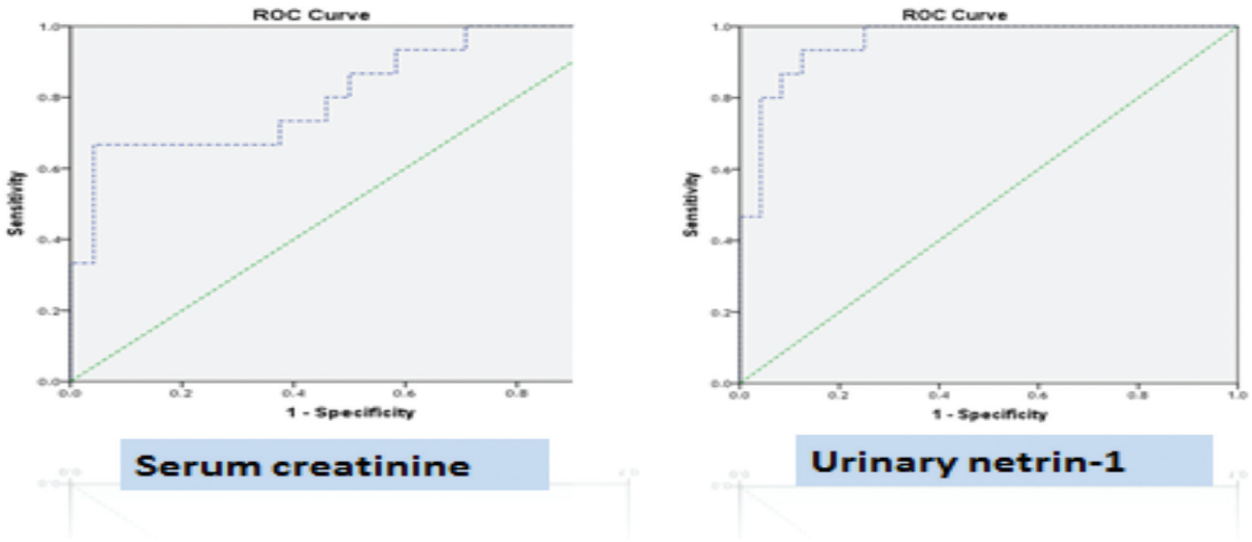
CPB, cardiopulmonary bypass; Hb, hemoglobin.

Table 6 Validity of the studied biomarker as a predictor for acute kidney injury 6 h after cardiopulmonary bypass

Biomarker	Sensitivity	Specificity	PPV	NPV	*P*
Urinary netrin-1 6 h after CPB (pg/ml)	86.7%	91.7%	86.6%	91.6%	<0.001**
Serum creatinine (mg/dl)	53.3%	66.7%	50%	69.5%	0.21
Combined urinary netrin and serum creatinine	86.7%	91.7%	86.6%	91.6%	<0.001**

Urinary netrin-1 (pg/ml) 6 h after CPB cutoff=107.3 (pg/ml). CPB, cardiopulmonary bypass; NPV, negative predictive value; PPV, positive predictive value.

Figure 1



Receiver operating characteristic (ROC) curve of serum creatinine and urinary netrin-1 at 6 h after cardiopulmonary bypass surgery.

Ramesh *et al.* [3] have found that using serum creatinine, AKI was detected on almost 48 h after CPB, though urine Ntn1 increased early, peaked at 6 h, and remained elevated up to 48 h after surgery. The predictive power of Ntn1 as demonstrated by the area under the receiver-operating characteristics curve for the diagnosis of AKI at 6 and 12 h after CPB was 0.86, and 0.89, respectively.

Reeves *et al.* [10] have analyzed urinary Ntn1 excretion after ischemia-reperfusion, cisplatin, folic acid, and endotoxin-induced renal injury in mice. They found significant increase of urinary Ntn1 levels within 3 h of ischemia-reperfusion, reached a peak level at 6 h, and decreased later, returning to near baseline by 72 h. However, a significant change in serum creatinine appeared after 24 h of reperfusion. By immunohistochemical localization, Ntn1 was highly expressed in tubular epithelial cells in transplanted human kidneys. In 2010, Rameshand colleagues have studied urinary Ntn1 levels by sandwich ELISA in urine samples from patients with AKI of different causes. They found urinary Ntn1 levels to have increased in all AKI groups [16]. The highest values were observed in patients with sepsis and in transplant patients immediately postoperatively [17].

Our study demonstrated a statistically significant correlation between 6 h urinary Ntn1 and CPB time, whereas no statistically significant correlation between 6 h urinary Ntn1 versus each of age (years), serum creatinine 6 h after CPB (mg/dl), estimated glomerular filtration rate 6 h after CPB, hemoglobin (g/dl), in the AKI group. The 24 h urinary Ntn1 was significantly correlated with both CPB time and serum creatinine 24 h after CPB in the AKI group. Adjusting for CPB time, the 6-h Ntn1 remained a powerful independent predictor of AKI, with an odds ratio of 1.20 (95% confidence interval: 1.08–1.41; P=0.006) [3].

The sensitivity and specificity of urinary Ntn1 to detect AKI at 6 h after CPB surgery was 86.7 and 91.7%, respectively, at a cutoff value of 107.3 pg/ml. Combined urinary Ntn1 and serum creatinine had the same sensitivity and specificity. In comparison to Ramesh *et al.* [3] study on pediatric patients, the sensitivities and specificities for three Ntn1 concentrations obtained at the 6-h time point correspond to 95% sensitivity, optimal sensitivity and specificity, and 95% specificity. The optimal combination of sensitivity (84%) and specificity (80%) yielded at 6 h after cardiac surgery. This study confirmed that urinary Ntn1 is an accurate predictor of AKI as early as 6 h after CPB surgery, but we have not measured serum Ntn1 levels in this cohort of patients to determine whether the Ntn1 level in serum has a predictive value for AKI.

In conclusion, urinary Ntn1 might be considered as an early sensitive biomarker of acute kidney injury at 6 h after CPB surgery instead of serum creatinine that rise only 24 h after CPB surgery in patients with CSA-AKI.

The limitation to our study is its relatively small sample size. Thus, these results will need to be validated in a larger population, including patients

with the usual confounding variables and comorbid conditions.

Second, our study was a cohort with normal kidney function, and it will be important to confirm our findings in documented high-risk settings, such as a preexisting kidney dysfunction, diabetes mellitus, volume depletion, concomitant nephrotoxic drug use, and hemodynamically compromised patients.

Third, in addition to Ntn1, simultaneous examination of other urinary biomarkers as potential predictors of AKI may provide additional benefit.

In addition to information on Ntn1, simultaneous examination of other urinary biomarkers as potential predictors of AKI may provide additional information.

Recommendations

To use urinary Ntn1 at 6 h after CPB surgery as an early biomarker of CSA-AKI.

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Conflicts of interest

There are no conflicts of interest.

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