Effect of intrarenal renin-angiotensin-aldosterone system on renal function in patients after cardiac surgery

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Abstract
The aim of the study was to investigate the influence of intrarenal RAS on the decrease of renal function in patients undergoing cardiac surgery with cardiopulmonary bypass. This observational study investigated the activation of intrarenal RAS in 24 patients with AKI after cardiac surgery with cardiopulmonary bypass. The activation of intrarenal RAS was determined by urinary angiotensinogen (uAGT), which was measured at 12 hours before surgery, 0 and 12 hours after surgery. The results were compared with those of 21 patients without AKI after cardiac surgery with cardiopulmonary bypass. Clinical and laboratory data were collected. Compared with baseline, all patients with cardiac surgery had activation of intrarenal RAS at 0 and 12 hours after surgery. The activation of intrarenal RAS was found significantly higher at both 0 and 12 hours after surgery in AKI group versus non AKI group (6.18 ± 1.93 ng/mL vs 3.49 ± 1.71 ng/mL, 16.38 ± 7.50 ng/mL vs 6.04 ± 2.59 ng/mL, respectively). There was a positive correlation between the activation of RAS at 0 hour after surgery and the decrease of renal function at 48 hours after surgery (r² = 0.654, P = .001). These findings suggest that uAGT might be a suitable biomarker for prediction of the occurrence and severity of AKI after cardiac surgery. Inhibition of intrarenal RAS activation might be one the path of future treatment for this type of disease.

Abbreviations: ACE = angiotensin converting enzyme, AGT = angiotensinogen, AKI = acute kidney injury, CPB = cardiopulmonary bypass, IL-18 = interleukin-18, IL-6 = interleukin-6, IL-8 = interleukin-8, KDIGO = Kidney Disease Improving Global Outcomes, KIM-1 = kidney injury molecule 1, NAG = N-acetyl-glucosaminidase, NGAL = neutrophil gelatinase-associated lipocalin, RAS = renin angiotensin aldosterone system, uAGT = urinary angiotensinogen.

Keywords: AKI, cardiac surgery, cardiopulmonary bypass, RAS, uAGT

1. Introduction
Cardiac surgery performed with cardiopulmonary bypass (CPB) is a common surgical procedure worldwide.[1] However, it does have drawbacks: the patient’s organs are subjected to ischemia-reperfusion (I/R) injury, which can leads to acute kidney injury (AKI), as renal ischemia is an important mechanism of the occurrence of AKI. There is growing recognition that the renin angiotensin aldosterone system (RAS) plays an important regulatory role in kidney damage and the abnormal activation of RAS will lead to further aggravation of renal ischemia.[2,3]

Existing evidence suggests that local RAS are present in kidneys as well as in the circulatory system.[4] Renin is secreted by juxtaglomerular cells and angiotensin converting enzyme (ACE) is present in the proximal tubule, distal tubule, and collecting duct.[5] In recent years, researchers have identified angiotensinogen (AGT) mRNA and protein in renal proximal tubule cells. In consideration of the molecular size of AGT and protein binding characteristics, cyclic AGT cannot be filtered by glomerular filtration, indicating that urinary angiotensinogen (uAGT) mainly comes from the proximal renal...
2. Methods

2.1. Patients and study design

Consecutive patients undergoing cardiac surgery with cardiopulmonary bypass were included in this study from May 2018 to January 2019, which were all elective surgery. Patients were not eligible if they were <18 years of age, treated with renin angiotensin system inhibitor, with signs of active infection, or exposure to arterial contrast in the previous 4 weeks. The protocol shown above was approved by the Clinical Research Ethics Committee of the Henan Provincial People’s Hospital, the reference number is 2019-011-01 and informed consent was obtained from all participants according to the Declaration of Helsinki.

The usual clinical and analytical variables were recorded at baseline in all patients. Urine samples from patients were immediately frozen at −80°C to assay the level of uAGT. The study’s observational end point was the occurrence of AKI in 48 hours, evaluated as described in Table 1 after KDIGO guidelines.\(^1\)

2.2. Assay of uAGT

Concentrations of uAGT were measured by enzyme-linked immunosorbent assay (ELISA) using reagent kits from Elabscience (E-EL-H0300c) as previously reported.\(^1\)

2.3. Statistical analysis

The $t$ test was used to compare the differences in continuous variables. Medians and proportions were compared using Mann–Whitney $U$ and Fisher Exact tests, respectively. Categorical data are expressed as frequencies and percentages, and continuous data are expressed as mean and standard deviation or median and interquartile ranges (IQR). ANOVA was used to compare the differences in more than two groups of data. A $P$-value of <.05 was considered significant for all tests. All calculations were carried out with SPSS version 22.0 (SPSS, IL).

3. Results

3.1. Patient characteristics

A total of 45 consecutive patients who met the inclusion criteria and underwent surgery from May 2018 to January 2019 were included. Patient characteristics are shown in Table 2. In all, 40% of the study population were active smokers and 25% had a history of previous surgery. In AKI group, 45.8% had hypertension, and 12.5% had diabetes mellitus, and the data of non-AKI group were 47.6% and 19%, respectively. Compared with baseline, the level of uAGT at 0 and 12 hours after surgery in

### Table 1

Kidney disease improving global outcomes (KDIGO) classification.

| Stage | SCr | UO |
|-------|-----|----|
| 1     | Increase in SCr > 0.3 mg/dL (≥26.5 μmol /L) or increase in SCr > 150%–200% (1.5–1.9 x) | <0.5 mL/kg/h (6 h) |
| 2     | Increase in SCr > 200%–300% (≥2–2.9 x) | <0.5 mL/kg/h (12 h) |
| 3     | Increase in SCr > 300% (≥30) or Increase in SCr to ≥4 mg/dL (≥353.6 μmol /L) or initiation of renal replacement therapy | <0.3 mL/kg/h (24 h) or anuria (12 h) |

SCr = serum creatinine; UO = urine output.

### Table 2

Comparison of clinical indicators between AKI group and non-AKI group.

| Descriptive variables | AKI group (n=24) | Non-AKI group (n=21) | t/Z | P for trend |
|-----------------------|------------------|----------------------|-----|------------|
| Age, years            | 55.79±10.82      | 54.43±10.90          | 0.42| .68        |
| Male, %               | 50               | 61.9                 | 1.12| .29        |
| Body surface area (m²)| 1.64±0.18        | 1.65±0.20            | −0.15| .88       |
| Hypertension, %       | 45.8             | 47.6                 | 0.44| .64        |
| diabetes mellitus, %  | 12.5             | 19                   | 1.31| .19        |
| Left ventricular ejection fraction (EF, %) | 58.39±9.4 | 57.95±7.52 | 0.168 | .867 |
| Preoperative red blood cell count (×10¹²/L) | 4.53 (4.16,4.97) | 4.54 (4.04,4.84) | 0.41 | .68 |
| Preoperative hemoglobin (g/L) | 131.21±15.44 | 127.19±19.06 | 3.44 | .44 |
| Preoperative SCr (mmol/L) | 66.09±15.47 | 67.33±14.24 | 0.28 | .78 |
| Preoperative ProBNP (Pg/ml) | 589.00 (273.75,1625.50) | 341.00 (174.00,1435.00) | −0.89 | .36 |
| Preoperative serum lactate dehydrogenase (U/L) | 210.00 (184.00,235.30) | 150.00 (128.00,196.50) | 3.23 | .001 |
| Bleeding volume during operation (mL) | 300.00 (200.00,300.00) | 300.00 (200.00,300.00) | −0.01 | .99 |
| Intraoperative blood transfusion volume (mL) | 587.50 (425.00,1537.50) | 460.00 (337.50,1047.50) | 1.85 | .07 |
| Extracorporeal circulation time (min) | 192.46±109.58 | 154.57±77.35 | 1.32 | .19 |
| Aortic crossclamp time (min) | 106.50 (64.50,156.75) | 91.00 (57.50,125.50) | −0.92 | .36 |
| Change value of Scr at 48 h after operation (μmol/L) | 51.33±31.15 | 11.00±11.10 | 5.51 | <.001 |
| Scr at 48 h after surgery (μmol/L) | 104.54±40.24 | 75.33±15.02 | 3.137 | .003 |

Data are expressed as mean± standard deviation, n (%), or median (interquartile range).

AKI = acute kidney injury; EF = ejection fraction; SCr = serum creatinine.
all patients with cardiac surgery was significantly higher, as shown in Figure 1 and Figure 2. There were 24 patients (53.3%) who developed AKI during follow-up, here called the AKI group. The activation of intrarenal RAS at 0 and 12 hours after cardiac surgery in AKI group was significantly higher than that in the non-AKI group (Table 3). There was a positive correlation between the activation of intrarenal RAS at 0 hour after surgery and the decrease of renal function at 48 hours after surgery ($r = 0.654$, $P = .001$).

4. Discussion

Previous studies have shown that the incidence of AKI after cardiac surgery is about 40%, which is a common reason to prolong the hospitalization time of patients. This retrospective study was designed to evaluate the influence of intrarenal RAS in the development of AKI in patients undergoing cardiac surgery with cardiopulmonary bypass. We demonstrated that the occurrence of AKI after cardiac surgery implies an activation of intrarenal RAS. This is a major breakthrough in understanding the underlying mechanisms of this frequent disease.

Our results indicated that the incidence of AKI is much higher than has been reported in previous studies. The reason might be that cardiac surgery can cause much longer periods of renal ischemia than other surgery.

Extensive experimental and clinical studies have implicated the RAS in the progression of renal injury. Our results indicated that the activation of intrarenal RAS was much higher in patients clinically diagnosed with AKI after cardiac surgery than that in the non-AKI group, which means that the activation of intrarenal RAS plays an important role in the development of AKI after cardiac surgery. Activation of RAS not only aggravates intraglomerular hyperfiltration and subsequent glomerular hypertrophy in its early phase but also contributes to the progression of glomerulosclerosis and fibrosis in the later phase of kidney disease. These results showed the importance of identifying uAGT in patients after cardiac surgery and might provide a rationale for a distinct therapeutic management to prevent AKI in uAGT-positive patients.

We also found that there was a positive correlation between the activation of intrarenal RAS at 0 hour after surgery and the decrease of renal function ($r = 0.654$, $P = .001$), indicating that uAGT may serve as a predictive biomarker of the occurrence and severity of AKI after cardiac surgery. A previous study showed that uAGT can indicate the long-term prognosis of acute kidney injury in mice. Although uAGT at 0 hour after surgery was related to the severity of AKI, the development of AKI was

| Time points       | AKI group (n=24) | Non-AKI group (n=21) | t/Z       | P for trend |
|-------------------|------------------|----------------------|-----------|------------|
| 12 h before surgery | 1.64 ± 0.80 (ng/mL) | 1.91 ± 0.83 (ng/mL) | −1.099 | .278       |
| 0 h after surgery | 6.18 ± 1.93 (ng/mL) | 3.49 ± 1.71 (ng/mL) | 4.941 | .000       |
| 12 h after surgery | 16.38 ± 7.50 (ng/mL) | 6.04 ± 2.59 (ng/mL) | 6.002 | .000       |

Data are expressed as mean ± standard deviation. $P < .05$ was considered significant.

AKI = acute kidney injury; uAGT = urinary angiotensinogen.
affected by many factors. We will further explore whether uAGT was an independent risk factor affecting the occurrence and progression of AKI.

The limitations of this study are the following: recently, potential urinary and serum biomarkers of AKI have been identified, such as cystatin-C, neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-glucosaminidase (NAG), kidney injury molecule 1 (KIM-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin 18 (IL-18). Unlike these biomarkers, the sensitivity of uAGT for diagnosis of AKI after cardiac surgery with cardiopulmonary bypass is not clear. Second, we also acknowledge the limitations inherent in any observational study design. Nevertheless, the results concerning the effect of uAGT on the study end point and clinical complications offer solid evidence implicating uAGT as a causative factor of complications in patients undergoing cardiac surgery with cardiopulmonary bypass.

In conclusion, our results provide new insights into the pathophysiology mechanisms underlying intrarenal RAS activation in AKI after cardiac surgery. Inhibition of intrarenal RAS activation might be a suitable direction for future treatment for this type of disease, and uAGT might be a predictive biomarker of occurrence and severity of AKI.

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