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Correlation between angiotensin and acute kidney injury in patients with sepsis

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Abstract. – OBJECTIVE: The aim of the study was to analyze the changes in angiotensin (Ang) levels in patients with sepsis complicated with acute kidney injury (AKI) and evaluate the relationship between Ang and AKI.

PATIENTS AND METHODS: Prospective research methods were used in this study. A total of 66 sepsis patients admitted to the Intensive care Unit (ICU) of the First Hospital of Hebei Medical University from October 2020 to January 2021 were enrolled. According to the occurrence of AKI, patients were divided into the sepsis-associated AKI (SA-AKI) group and the non-AKI group. The levels of Ang-1 and Ang-2 were compared between the two groups. The relationship between Ang and glomerular filtration rate (GFR) in sepsis patients was studied by correlation analysis.

RESULTS: Plasma Ang-1 in the SA-AKI group was significantly higher than that in the non-AKI group (0.39±1.05 ng/ml *vs*. 0.10±0.24 ng/ml, *p*=0.039). The Ang-2/Ang-1 in the SA-AKI group was lower than that in the non-AKI group with a significant difference (52.55±191.38 *vs*. 349.50±327.49, *p*=0.001). Correlation analysis indicated that Ang-1 was negatively correlated with GFR (r=-0.12, *p*=0.031), while Ang-2/Ang-1 was positively correlated with GFR (r=0.21, *p*<0.001). The Ang-2 was positively correlated with GFR (r=0.204, *p*<0.001)

CONCLUSIONS: Plasma Ang-1 and Ang-2 levels are suggestive for assessing the risk of AKI in patients with sepsis.

Key Words:
Sepsis, Acute kidney injury, Angiotensin, Endothelial dysregulation.

Abbreviations

AKI: Acute kidney injury; Ang: angiotensin; GFR: Glomerular filtration rate; ICU: Intensive Care Unit; KDIGO: Kidney Disease: Improving Global Outcomes; NGAL: neutrophil gelatinase-associated lipocalin; RAS: renin-angiotensin system; SA-AKI: sepsis-associated acute kidney injury; Scr: serum creatinine; sCys: C serum Cystatin C; SOFA: Sequential organ failure.

Introduction

Sepsis is a life-threatening organ dysfunction caused by dysregulation of the body's response to infection[1]. Sepsis often causes multiple organ dysfunction, and the kidney is one of the most frequently involved organs. The incidence and mortality of patients with sepsis-associated acute kidney injury (SA-AKI) are both high and the prognosis is poor[2]. Acute kidney injury (AKI) refers to a clinical syndrome caused by a variety of causes and a sudden decline in renal function within a short period. The clinical manifestations are increased serum creatinine (Scr) level, decreased urine output, and the need for kidney replacement therapy[3]. AKI is the early stage of renal insufficiency; its occurrence and development may lead to an increased risk for heart failure and other serious consequences[4]. Considering that patients with SA-AKI have significantly increased hospital stays, medical costs, and mortality compared to patients without AKI, the early diagnosis of SA-AKI is particularly critical, and the current diagnostic criteria for SA-AKI, which rely on Scr and urine volume, have certain clinical limitations[2]. The new biomarkers will compensate for the shortcomings of early diagnosis.

Previous studies[5,6] on the early recognition of SA-AKI biomarkers have reported neutrophil gelatinase-associated lipocalin (NGAL) and serum Cystatin C (sCys C). For NGAL, inflam-

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mation in sepsis leads to increased lung and liver synthesis, reducing the specificity of this marker for kidney injury[7]. In addition, the current method of sCysC detection lacks a standard, which still needs to be further solved. Scholars[8] have shown that impaired microvascular endothelial and epithelial cell function plays an important role in renal ischemia-reperfusion injury. Studies[9] reported that dysregulation of the angiotensin (Ang) molecular pathway in patients with sepsis was involved in endothelial overactivation. The Ang is divided into Ang-1 and Ang-2, which play an important role in the verification of vascular endothelium[10]. Meanwhile, the renin-angiotensin system (RAS) of sepsis patients is overactivated[11], suggesting that Ang, as an endothelial marker, may be involved in the occurrence and development of AKI. Currently, there are few studies on whether Ang can be used as an early diagnostic indicator of SA-AKI. Therefore, this study aimed to analyze the plasma Ang level in patients with SA-AKI and evaluate the correlation between Ang and the occurrence of SA-AKI.

Patients and Methods

Study Patients

In this prospective study, a total of 66 sepsis patients admitted to the Intensive Care Unit (ICU) of the First Hospital of Hebei Medical University from October 2020 to January 2021 were included consecutively. Sepsis patients were divided into the SA-AKI group and non-AKI group according to whether AKI occurred during ICU hospitalization. The diagnosis of AKI was based on the Kidney Disease: Improving Global Outcomes (KDIGO) diagnostic criteria[3]. These criteria state that the level of SCr should be ≥0.3 mg/dL (≥26.5 μmol/L) or 1.5 times or more the basal value within 48 h, or within 7 days.

Inclusion criteria: (1) Patients older than 18 years of age; (2) Patients with sepsis who meet the "international sepsis guidelines criteria"[12].

Exclusion criteria: (1) Patients with autoimmune disease, malignancy, or recent immunosuppressive therapy; (2) Patients with chronic kidney disease or severe renal impairment before ICU admission; (3) Patients who had recently received continuous or intermittent blood purification therapy; (4) Patients receiving antihypertensive drugs such as Ang converting enzyme inhibitors or Ang-2 receptor antagonists; (5) Patients who have taken medications that affect the renin-angiotensin-aldosterone system prior to admission; (6) Maternal status.

All protocol was designed in accordance with the Helsinki Declaration. This study was approved by the Ethics Committee of the First Hospital of Hebei Medical University (No. 20200655), and the informed consent forms were obtained from all patients.

Collection of Blood Samples

Venous blood samples were collected daily during hospitalization and ICU admission. Before collection, the anticoagulant tube (Tuoren Medical Technology Co., LTD., Shenzhen, China, Product No. 20172410092) stored at -4°C was naturally placed at room temperature (18-25°C). In the morning, the venous blood of patients and that of the control group in fasting, supine position, and resting state were taken. After collection, the blood and anticoagulant were mixed gently upside down three times and sent to the laboratory within 20 minutes. The samples were centrifuged at 4,000 r/min for 10 min. Then, the supernatant plasma was taken, and the regulator was added in the ratio of 8:1. 100 μL PH buffer was added to 800 μL plasma and mixed well.

Measurement of Ang levels and Glomerular Filtration Rate (GFR)

Plasma Ang-1 and Ang-2 concentrations were determined by chemiluminescence. The Ang kit was inserted into the reagent chamber of the automatic chemiluminescence analyzer (Maglumi 4000 Plus, Shenzhen New Industry Biomedical Engineering Co., LTD., Shenzhen, China), and balanced on the instrument for 30 minutes before use to make the magnetic microspheres fully suspended before the test. Then, it was calibrated by measuring the high and low point calibrators and adjusting the pre-defined kit main curve to a new instrument-specific operating curve. Quality control was carried out with a kit supporting quality control products. The kit for Ang determination was produced by Shenzhen New Industry Biomedical Engineering Co., LTD. (No. 20000003). The specimen was loaded into the instrument, the sample information was inputted, and the items were tested. The calibration detection signal was used to adjust the main curve of the entire storage to the working curve, and the instrument automatically calculated the concen-

Table I. Comparison of the general situation of two groups of patients.

Index	AKI group	non-AKI group	*p*-value
Gender (n, %)			0.799*
Male	30 (73.2)	19 (76.0)	
Female	11 (26.8)	6 (24.0)	
Age	65.61 ± 15.06	65.88 ± 14.237	0.778#
Hypertension			0.853*
Positive	19 (46.3)	11 (44.0)	
Negative	22 (53.7)	14 (56.0)	

*Compared by Chi-square test; #Compared by Student's *t*-test. Acute kidney injury (AKI).

tration of analyte in each sample with the help of the working curve and the detection signal of the analyte in the sample.

A total of 3 mL of venous blood was collected from patients 2 h, 24 h, and 48 h after admission and placed in a sodium citrate vacuum anticoagulant tube, centrifuged at 3,500 r/min for 5 min. Upper plasma was collected, Scr level was detected by sarcosine oxidase method, and GFR was calculated[13].

Sequential organ failure (SOFA)[14] score and acute physiology and chronic health evaluation II (APACHE-II) score[1] were used to evaluate the condition of sepsis. The sepsis patients included in the study received fluid resuscitation, anti-infection, mechanical ventilation, and vasoactive drugs. For Sepsis patients who needed renal replacement therapy, bedside hemofiltration therapy was carried out according to appropriate protocols.

Statistical Analysis

Statistical analysis was performed using the SPSS software program (version 22.0; IBM Corp., Armonk, NY, USA). Normally distributed measurement data were expressed as mean ± standard deviation (SD), and the comparisons were examined by the Student's *t*-test. Pearson's correlation was used to evaluate the correlation between Ang and GFR. The test level α was 0.05 on both sides. $p<0.05$ was considered statistically significant.

Results

This study included 66 sepsis patients. There was no statistical difference in the general situation between the two groups (Table I). The Ang-1 in the SA-AKI group was significantly higher than that in the non-AKI group ($p=0.039$). The Ang-2/Ang-1 in the SA-AKI group was lower than that in the non-AKI group, with a significant difference ($p=0.001$) (Table II). Correlation analysis indicated that Ang-1 was negatively correlated with GFR (r=-0.12, $p=0.031$), while Ang-2/Ang-1 was positively correlated with GFR (r=0.21, $p<0.001$). The Ang-2 was positively correlated with GFR (r=0.204, $p<0.001$). Compared with the non-AKI group, the Ang-1 level was higher and increased, while Ang-2/Ang-1 was lower and decreased in the SA-AKI group (Figure 1).

Discussion

Sepsis refers to a series of uncontrolled inflammatory reactions caused by an infection in the body, leading to life-threatening organ dysfunction[1]. It is common in critically ill patients, and the form of the disease is changeable. Given the high mortality rate, prompt intervention is crucial for clinicians to make quick judgments and administer treatment. Sepsis induces the activation of white blood cells and endothelial cells during the onset of the disease, further

Table II. Comparison of Ang levels between SA-AKI and non-AKI groups.

Group	Ang-1 (ng/ml)	Ang-2 (ng/ml)	Ang-2/Ang-1
SA-AKI group	0.39 (1.05)	38.93 (43.92)	52.55 (191.38)
Non-AKI group	0.10 (0.24)	47.67 (31.94)	349.50 (327.49)
p	0.039	0.078	0.001

Ang, angiotensin; SA-AKI, sepsis associated acute kidney injury; AKI, acute kidney injury.

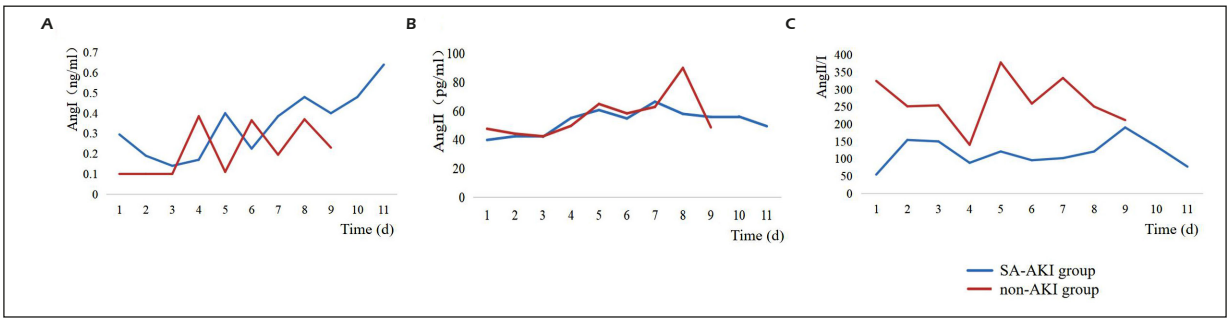

Figure 1. Line chart of angiotensin (Ang) level changes in sepsis patients. **A**, Plasma Ang-1 in sepsis-associated acute kidney injury (SA-AKI) group was higher than that in the non-AKI group and showed an increasing trend; **B**, There was no significant difference in plasma Ang-2 between the SA-AKI group and the non-AKI group; **C**, the Ang-2/Ang-1 was lower and decreased in SA-AKI group than that in the non-AKI group.

mediates the inflammatory response and releases inflammatory factors. This cascade effect will lead to cell and organ damage[15]. Previous reports[16,17] have described the body's response to infection as an "inflammatory cytokine storm", which activates various neuroendocrine mechanisms, including the stress response. The kidney is one of the common target organs of severe sepsis with high incidence and poor prognosis. It is reported[2] that SA-AKI can be an independent risk factor affecting the prognosis of patients, with a fatality rate of more than 60%. Therefore, early identification of SA-AKI can facilitate timely intervention to reduce the incidence of AKI in sepsis patients.

During the development of sepsis, the activation of the RAS system plays an important role in the progression of the disease. Overactivated RAS may further aggravate inflammatory response and disrupt homeostasis. During this process, organs such as kidneys and lungs are most susceptible to inflammatory responses, resulting in dysfunction[18]. Due to the abnormal distribution of circulating blood volume in patients with sepsis, effective circulating blood volume is reduced, which reduces the blood flow of important organs in the body, especially the kidney, and leads to continuous hypoperfusion, thus causing organ dysfunction. Therefore, AKI, as a common complication of sepsis patients, may be related to the increase in RAS level. Angiotensin-converting enzyme inhibitors (ACEI) and Ang-2 receptor antagonists are commonly used to block RAS. Some studies[19] have pointed out that systemic angiotensin-converting enzyme (ACE) is associated with basal blood pressure regulation and normal kidney development, and renal ACE seems to be related to the pathophysiology of hypertension. Current clinical detection methods cannot distinguish between the two receptors. Therefore, the role of ACE in sepsis-related AKI was not discussed in this study. In patients with type 1 cardiorenal syndrome, the use of ACEI or Ang-2 receptor antagonists was not associated with improved renal function after 72 hours[20]. At the same time, considering that ACEI inhibits the conversion of Ang-1 to Ang-2 by inhibiting ACE, this mechanism may interfere with the Ang level in patients with septic AKI. Therefore, patients who were treated with ACEI or Ang-2 receptor antagonist antihypertensive drugs were not included in this study.

Activation of endothelial cells, destruction of vascular barrier, and microvascular leakage are important causes of organ dysfunction in patients with sepsis[21]. In the kidney, the vascular endothelial barrier consists of endothelial cells, polyglycosomes, basement membrane, and podocytes. The destruction of the vascular barrier plays an important role in sepsis complicated with AKI[22]. In sepsis, the "storm of inflammatory factors" will destroy the connection between cells, causing endovascular endothelial cell calcification to take place and further induce endothelial cell leakage, resulting in the integrity of the vascular barrier damage, which is more serious in SA-AKI[23]. In this process, Ang-1 and Ang-2 regulate vascular permeability by competitively binding to Tie2 receptors on endothelial cells[24]. Decreased arterial blood pressure in patients with sepsis stimulates the sympathetic nervous system and increases RAS excitability, thereby increasing Ang secretion[25]. In this study, the plasma Ang-1 level of patients with SA-AKI was higher than that of patients without AKI, while the plasma Ang-2/Ang-1 level of patients with SA-AKI was lower than

that of patients without AKI, suggesting that the plasma Ang-1 or Ang-2/Ang-1 level of patients with sepsis is of certain value in predicting the occurrence of AKI. Correlation analysis showed that Ang-1 was negatively correlated with GFR, Ang-2/Ang-1 was positively correlated with GFR, and Ang-2 was not significantly correlated with GFR, which was consistent with the results of Ye et al[26]. These results indicated that the increase of Ang-1 and the decrease of Ang-2/Ang-1 suggested that the GFR and renal function of sepsis patients decreased.

Limitations

This study also had the following limitations: the first is the limited sample size of the study. The levels of the biological factors involved in this study were not dynamically observed. Secondly, the cases included in this study were from a single center, so there may be some bias in the selection of patients. Thirdly, the follow-up period is relatively short. Fourth, there was no comparison between Ang and other biomarkers in this study. Fifth, there are other factors/confounders that may impact serum angiotensin levels, such as patients' history of ACEI drugs. Therefore, a more scientific sample size and more comprehensive design are still needed to improve the quality of research results.

Conclusions

Increased Ang-1 and decreased Ang-2/Ang-1 in sepsis patients suggest a higher risk of developing AKI. Plasma Ang level is important for assessing the risk of AKI in sepsis patients and provides a clinical basis for timely treatment and intervention.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethics Approval

The study protocol was approved by the Ethics Committee of The First Hospital of Hebei Medical University (No. 20200655) and designed in accordance with Helsinki Declaration.

Informed Consent

Written informed consent was obtained from all the study subjects before enrollment.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Authors' Contribution

Chongbo Du and Xiufen Yang contributed to the conception and design of the study; Yuxiao Zhang, Peng Lu, Zhitao Zhao and Rui Du performed the experiments, collected and analyzed data; Dongliang Li, Chongbo Du and Xiufen Yang wrote the manuscript; Chongbo Du, Xiufen Yang and Ye Liu revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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