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ORIGINAL ARTICLE

Th17/Treg Imbalance in Acute Kidney Injury

Xiao Zhou, Guoqiang Bai, Meili Duan

Department of Critical Care Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing, China

SUMMARY

Background: **Helper T cell 17 (Th17) and regulatory T cells (Treg) play an important role in the inflammatory response. However, the role of Th17/Treg imbalance in acute kidney injury is not yet established. The aim of the study was to analyze Th17/Treg imbalance in acute kidney injury caused by sepsis or other reasons.**
Methods: **An observational prospective study was conducted. We enrolled adult patients admitted to the intensive care unit (ICU) with acute kidney injury caused by sepsis or other reasons and then followed up until 28 days or discharge. Healthy volunteers were followed during the same period as the control group. We investigated the differences in renal injury markers and inflammatory indicators between acute kidney injury (AKI) patients and the control group. The clinical data and peripheral blood samples of all patients were collected immediately after enrollment. An analysis of the data was conducted to determine if the Th17/Treg ratio could serve as a predictive marker of sepsis induced acute kidney injury (SAKI) in AKI patients.**
Result: **A total of 104 AKI patients were enrolled in the study, including 60 SAKI, 44 AKI without sepsis, while 10 healthy volunteers served as the control group. Infections, especially thoracoabdominal infection leading to sepsis, were the major cause of AKI in the study population (58%). Th17/Treg ratio, the proportion of Th17 cells, the concentration of interleukin-10 (IL-10), and the concentration of interleukin-17 (IL-17) of AKI patients showed a significant increase compared to that in the control group. The proportion of Th17 cells and Treg cells as well as the Th17/Treg ratio of the SAKI group were higher than those of the AKI without sepsis group. Chronic kidney disease (CKD) and Th17/Treg ratio were independent risk factors for SAKI. The AUC demonstrated that the Th17/Treg ratio measured 0.775 (95% CI 0.683 - 0.851, $p < 0.0001$). The cutoff value of Th17/Treg ratio for predicting SAKI was 0.033. When the Th17/Treg ratio was > 0.033, the sensitivity of predicting SAKI was 0.967, and the specificity was 0.500. The 28-day mortality and renal function recovery rate between the SAKI group and the AKI without sepsis group did not differ.**
Conclusions: **There was an imbalance of Th17/Treg in acute kidney injury. Compared with AKI caused by other factors, Th17/Treg ratio was higher in SAKI patients. There was no difference in 28-day mortality and renal function recovery rate among AKI patients with different etiologies.**
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Correspondence:
Meili Duan
Department of Critical Care Medicine
Beijing Friendship Hospital
Capital Medical University
No. 95 Yong'an Road
Xicheng District
Beijing
China
Phone: + 86 10-63138013
Email: dmeili@ccmu.edu.cn
ORCID: 0000-0001-5947-4938

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KEYWORDS

acute kidney injury, T helper 17 cells, regulatory T cells, imbalance, sepsis

LIST OF ABBREVIATIONS

Th17 - Helper T cell 17
Treg - Regulatory T cell
ICU - Intensive Care Unit
AKI - Acute Kidney Injury
KDIGO - Kidney Disease: Improving Global Outcomes

SAKI - Sepsis-induced Acute Kidney Injury
BMI - Body Mass Index
CKD - Chronic Kidney Disease
APACHE II - Acute physiology and chronic health evaluation
NAGL - Neutrophil gelatinase-associated lipocalin
Cr - Serum creatinine
BUN - Blood urea nitrogen
PCT - Procalcitonin
Lac - Lactic acid
OI - Oxygenation index
WBC - White Blood Cell
GR - Neutrophil granulocyte
LY - Lymphocyte
NLR - Neutrophil Lymphocyte Ratio
LOS - Length of hospital stay
EDTA - Ethylenediaminetetraacetic Acid
PBMCs - Peripheral Mononuclear Cells
ELISA - Enzyme-linked immunosorbent assay
IL-10 - Interleukin-10
IL-17 - Interleukin-17
TNF-α - Tumor necrosis factor alpha
RRT - Renal Replacement Therapy
TGF-β - Transforming Growth Factor-β
IQRs - Interquartile Ranges
ROC - Receiver operating characteristic curve
AUC - Area Under the Curve
CI - Confidence interval

INTRODUCTION

Acute kidney injury (AKI) is defined as acute and rapid deterioration of kidney function as a result of various etiologies [1]. The global burden of AKI-related mortality exceeds by far that of breast cancer, heart failure or diabetes [2], with mortality remaining high during the past 50 years. As the global concern, the large spectrum of AKI implies diverse pathophysiological mechanisms. The kidney is a highly vascular organ, vital for maintaining internal homeostasis via removal of toxins from the blood. Its anatomical structure and function render it vulnerable to a variety of immune, and nonimmune mediated injury [3]. Th17/Treg balance is regarded as a key factor in immune homeostasis [4]. The imbalance of Th17/Treg cells has been confirmed to be associated with sepsis and various inflammatory diseases [5,6]. Our previous study confirmed that Th17/Treg imbalance was associated with sepsis induced acute kidney injury (SAKI) and AKI severity [7]. It is thus fascinating to hypothesize that there is a Th17/Treg imbalance in AKI patients caused by different etiologies. Thus, given the clinical implications, it was decided to study peripherally circulating Th17/Treg in SAKI and AKI without sepsis patients.

MATERIALS AND METHODS

Design and setting

Our study was a prospective, observational, and single-center study conducted between January 2022 to December 2023. This study was in accordance with the Helsinki Declaration and approved by the Medical Ethics Committee of Beijing Friendship Hospital, Capital Medical University (No. 2021-P2-006-02).

Participants

The study population was divided into three arms, SAKI, AKI without sepsis, and healthy volunteers. Patients presented with acute kidney injury hospitalized in the department of critical care unit of Beijing Friendship Hospital were studied. The inclusion criteria included: 1) Age ≥ 18years old; 2) the definition of AKI was in accordance with the Acute Kidney Injury Working Group of KDIGO (Kidney Disease: Improving Global Outcomes) in 2012 [8]. The exclusion criteria contained the following items: 1) patients with autoimmune disease or HIV; 2) Patients with recent TB therapy (< 1 year), or treated with corticosteroids, immunosuppressive or antiretroviral therapy (ART). The control group was composed of healthy volunteers who visited the physical examination center of our hospital during the same period.

Sample collection and measurements

We recorded urine output for a 24-hour period and serum creatine of all candidates. A 5 ml sample of blood collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes was subjected to Ficoll gradient method for making a single cell preparation of peripheral blood mononuclear cells (PBMCs). The regulatory cell number was approximately 1 x 10^6 cells/mL. For flow cytometry, the cells were labeled with a monoclenal antibody conjugated to fluorescein (CD3 mAb, CD4 mAb, IL-17 mAb, CD25 mAb, CD127 mAb) (InvitrogenTM) with a flow cytometer (AttuneTM NxT, ThermoFisher Scientific). As Tregs CD3+CD4+CD25+ CD127- lymphocytes were considered according to Barrios-Angulo CE et al. [9]. The ratio of Th17/Treg (CD3+CD4+IL-17+/CD3+CD4+CD25+CD127-) lymphocytes in humans was determined using flow cytometry. Reports from the flow cytometer were received in the format given in Figure S1 and Figure S2. Serum samples were prepared immediately by centrifugation of peripheral venous blood. Neutrophil gelatinase-associated lipocalin (NAGL) and cytokines (interleukin-10, interleukin-17 and tumor necrosis factor alpha) were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's guidelines (Biolegend, USA). For the AKI patients, we collected the clinical data including medical history, APACHE II score, and laboratory investigations (blood urea nitrogen, procalcitonin, lactic acid, oxygenation index, white blood cell, neutrophil granulocyte, lymphocyte, neutrophil lymphocyte ratio, etc.).

The primary endpoint for this study was rate of renal recovery in AKI patients, with 28-day mortality, hospital length of stay (LOS), in ICU length of stay and expenses in the ICU as key secondary endpoints. Renal function recovery is defined based on the patient's creatinine concentration at discharge (or 28 days of follow-up). The patients in complete recovery group did not have acute kidney injury at discharge (the concentration of creatinine below 1.5 times baseline). Compared with AKImax, the reduction in staging of acute kidney injury is considered as partial recovery of renal function. For patients in AKI KDIGO3 stage who require renal replacement therapy (RRT) treatment, if they are discharged without renal replacement therapy but still diagnosed with acute kidney injury (even if they are still in AKI KDIGO3 stage), it is still considered as partial recovery of renal function. Patients with complete or partial recovery of renal function are defined as Recovery group. Persistent AKImax status (AKI KDIGO3 phase requiring RRT treatment) or exacerbation of AKI staging is defined as non-recovery group [10].

Statistical analysis

Continuous variables conforming to a normal distribution are presented as mean ± SD. Independent samples t-tests were used for comparisons between two groups. Continuous variables that did not conform to a normal distribution are presented as median values and interquartile ranges (IQRs). The Mann-Whitney U test was used for comparisons between two groups. The Kruskal-Wallis test was applied for multivariate analysis. Categorical data were summarized using number (percentage) and were compared using the chi-squared test. Binary univariate and multivariate logistic regression analyses were conducted for risk factor assessment. The area under the receiver operating characteristic (ROC) curve was calculated to evaluate the diagnostic and prognostic value of the tested parameters. Medcalc software for Z-test was used to evaluate the significant differences in AUC. Sensitivity analyses were performed in subjects of AKI patients without chronic kidney disease (CKD). All reported probability values are two tailed, and $p < 0.05$ was considered statistically significant. All missing data were excluded in the analysis. Analyses were performed using SPSS 25.0 software (IBM Corp., Armonk, NY, USA) and graphs were created by GraphPad Prism 8 (GraphPad, San Diego, CA).

RESULTS

We studied 104 patients with kidney injury, 60 with SAKI and 44 without sepsis. Ten healthy volunteers were included as controls. The rationale behind having a small size control group was to gain clarity regarding T-cell activity in health, i.e., to get an idea of Th17:Treg ratio in individuals without diabetes, hypertension, pre-existing kidney injury or any other risk factor or health ailment. Figure 1 illustrates causes of AKI in the study population.

Infections, especially thoracoabdominal infection leading to sepsis, were the major cause of AKI in our study population (58%), followed by dehydration induced nephropathy (15%). The other causes of AKI in our study were drug induced, obstructive uropathy, and cardio renal syndrome. More than 50% of patients with AKI belonged to stage 3 of the 2012 KDIGO criteria whereas only 9.6% of patients with AKI belonged to stage 1 and 36.5% belonged to stage 2.

Comparisons of AKI patients with healthy volunteers

Compared with healthy volunteers, indicators related to kidney damage of AKI patients including serum Cr, 24-hour urine output, and NAGL were found to be statistically significant ($p < 0.01$). The concentrations of sCr and NAGL were higher in AKI patients (either SAKI or AKI without sepsis) than in healthy volunteers. Similarly immune-related indicators including the proportion of Treg cells, the proportion of Th17 cells, concentration of IL-10, concentration of IL-17, and Th17/Treg ratio were significantly different among the healthy volunteers, SAKI patients, and AKI patients without sepsis. The proportion of Th17 cells and of IL-10 and IL-17 concentrations as well as the Th17/Treg ratio of AKI patients showed a significant increase compared to that in the control group. The concentrations of serum TNF-α did not differ. Further analysis found that as a biomarker of AKI and NAGL did not differ between SAKI group and AKI without sepsis group. However, The proportion of Th17 cells and Treg cells as well as Th17/Treg ratio of the SAKI group were higher than those of the AKI without sepsis group (Figure 2).

Comparisons of patients in the SAKI group and the AKI without sepsis group

To further analyze the role of Th17/Treg ratio in the different etiology of AKI groups, we divided AKI patients into two groups, the SAKI group and AKI without sepsis group (Table 1). The patients in the AKI without sepsis group were older than the SAKI group.

Gender distribution of the groups was not significantly different. CKD prevalence (40.9% vs. 16.7%, $p < 0.05$) and chronic cardiovascular diseases (68.2% vs. 43.3%, $p < 0.05$) were higher in the AKI without sepsis group, while diabetes (18.2% vs. 36.7%, $p < 0.05$) prevalence was lower than that in the SAKI group. In SAKI group, procalcitonin (PCT), Neutrophil granulocyte (GR), and Neutrophil Lymphocyte Ratio (NLR) were higher than in the AKI without sepsis group. The proportions of total T lymphocytes and CD4+ T lymphocytes were not significantly different between the two groups in our study. The proportion of Th17 cells in the peripheral blood of patients in the SAKI group was significantly higher than that in the AKI without sepsis group (0.14 vs. 0.08, $p < 0.001$) while the proportion of Treg cells was lower than that in the AKI without sepsis group (1.29 vs. 2.16, $p < 0.05$). Thus, compared with the AKI

Table 1. Comparison of clinical characteristics between SAKI and AKI without sepsis groups.

	Total (n = 104)	SAKI group (n = 60)	AKI without Sepsis group (n = 44)	Test value	p-value
Male [n (%)]	66 (63.5)	38 (63.3)	28 (63.6)	0.001	0.975
Age - year (median [Q1, Q2])	71.50 (56.75, 79.00)	67.50 (54.00, 76.00)	77.00 (64.00, 79.00)	-2.176	0.030 *
BMI - kg/m^2 (mean ± SD)	23.63 ± 4.66	23.44 ± 4.76	23.91 ± 4.56	-0.389	0.698
Comorbidities					
Hypertension [n (%)]	68 (65.4)	40 (66.7)	28 (63.6)	0.103	0.748
Diabetes [n (%)]	30 (28.8)	22 (36.7)	8 (18.2)	4.226	0.040 *
CKD [n (%)]	28 (26.9)	10 (16.7)	18 (40.9)	7.583	0.006 **
Chronic cardiovascular diseases [n (%)]	56 (53.8)	26 (43.3)	30 (68.2)	6.307	0.012 *
Chronic lung disease [n (%)]	12 (11.5)	10 (16.7)	2 (4.5)	3.654	0.056
Chronic liver disease [n (%)]	2 (1.9)	2 (3.3)	0 (0.0)	1.495	0.221
Nervous system disease [n (%)]	24 (23.1)	14 (23.3)	10 (22.7)	0.005	0.942
Rheumatic system disease [n (%)]	4 (3.8)	2 (3.3)	2 (4.5)	0.101	0.751
Malignant tumor [n (%)]	26 (25.0)	16 (26.7)	10 (22.7)	0.210	0.647
Physiological parameters					
APACHE II - score (median [Q1, Q2])	20.00 (15.25, 25.75)	20.50 (15.00, 25.00)	20.00 (17.00, 27.00)	-0.633	0.527
KDIGO Stage 1 [n (%)]	10 (9.6)	8 (13.3)	2 (4.5)	2.256	0.133
KDIGO Stage 2 [n (%)]	38 (36.5)	20 (33.3)	18 (40.9)	0.628	0.428
KDIGO Stage 3 [n (%)]	56 (53.8)	32 (53.3)	24 (54.5)	0.015	0.902
NAGL - ng/mL (median [Q1, Q2])	68.05 (59.83, 80.20)	74.10 (60.01, 80.94)	64.89 (59.77, 71.50)	-1.553	0.120
Urine output - L/24 hours (median [Q1, Q2])	0.96 (0.27, 2.414)	1.49 (0.39, 2.29)	0.47 (0.19, 1.91)	-2.277	0.023 *
Cr - µmol/L (median [Q1, Q2])	151.95 (103.63, 303.53)	127.00 (98.20, 206.10)	171.75 (121.70, 415.70)	-2.051	0.012 *
BUN - mmol/L (median [Q1, Q2])	13.62 (8.61, 19.80)	12.50 (8.45, 24.28)	13.93 (6.39, 30.95)	-0.395	0.693
PCT - ng/mL (median [Q1, Q2])	8.31 (1.36, 23.35)	19.51 (3.13, 44.24)	10.74 (3.79, 51.86)	-3.851	0.000 ***
Lac - mmol/L (median [Q1, Q2])	1.80 (1.20, 3.18)	1.95 (1.40, 3.93)	1.20 (1.13, 6.45)	-0.290	0.772
OI (median [Q1, Q2])	256.50 (172.75, 344.00)	348.00 (141.25, 506.50)	221.50 (163.75, 277.00)	-0.882	0.378
WBC - x 10^9/L (median [Q1, Q2])	11.16 (6.77, 17.90)	11.59 (7.38, 25.87)	9.62 (5.47, 13.91)	-1.921	0.055
GR - x 10^9/L (median [Q1, Q2])	10.29 (5.49, 16.54)	10.90 (5.99, 22.32)	8.50 (4.69, 11.80)	-2.211	0.027 *
LY - x 10^9/L (median [Q1, Q2])	0.70 (0.50, 1.08)	0.71 (0.25, 1.55)	0.66 (0.60, 0.86)	-0.487	0.626
NLR (median [Q1, Q2])	13.99 (7.45, 23.45)	17.73 (9.09, 43.69)	10.98 (7.82, 15.87)	-2.685	0.007 **
Immune indexes					
T lymphocyte ratio - % (median [Q1, Q2])	68.96 (60.06, 76.83)	69.54 (62.23, 76.26)	75.20 (62.38, 84.36)	-0.817	0.414
CD4+ T lymphocyte ratio - % (median [Q1, Q2])	39.83 (30.92, 50.68)	30.28 (22.87, 46.71)	47.17 (37.93, 54.36)	-0.757	0.449
The proportion of Treg cells - % (median [Q1, Q2])	1.45 (0.82, 2.88)	1.29 (0.59, 2.32)	2.16 (1.18, 3.27)	-2.587	0.010
The proportion of Th17 cells - % (median [Q1, Q2])	0.13 (0.08, 0.22)	0.14 (0.11, 0.24)	0.08 (0.04, 0.13)	-4.642	0.000

Table 1. Comparison of clinical characteristics between SAKI and AKI without sepsis groups (continued).

	Total (n = 104)	SAKI group (n = 60)	AKI without Sepsis group (n = 44)	Test value	p-value
Th17/Treg ratio (median [Q1, Q2])	0.09 (0.04, 0.19)	0.11 (0.07, 0.28)	0.04 (0.02, 0.09)	-4.734	0.000 ***
IL10 - pg/mL (median [Q1, Q2])	32.95 (17.57, 69.67)	38.13 (25.47, 109.01)	32.22 (11.98, 53.52)	-2.120	0.034 *
IL17 - pg/mL (median [Q1, Q2])	3.67 (1.55, 10.11)	4.89 (2.27, 12.18)	2.22 (1.36, 4.70)	-2.177	0.029 *
TNF-α - pg/mL (median [Q1, Q2])	9.16 (4.05, 13.38)	9.15 (4.05, 12.98)	9.90 (5.08, 47.36)	-1.057	0.290
General outcomes					
Renal recovery rate [n (%)]	66 (63.5)	42 (70.0)	24 (54.5)	2.615	0.106
28-day mortality [n (%)]	19 (18.4)	9 (15.3)	10 (22.7)	0.936	0.333
Hospital length of stay - days (median [Q1, Q2])	17.00 (8.00, 31.75)	14.00 (8.00, 32.00)	21.50 (8.00, 30.00)	-0.467	0.640
In ICU length of stay - days (median [Q1, Q2])	9.50 (6.25, 18.50)	9.00 (7.00, 17.00)	9.00 (2.00, 19.00)	-1.257	0.209
Expenses in ICU - ten thousand yuan (median [Q1, Q2])	7.90 (3.19, 16.87)	7.87 (4.04, 17.84)	7.24 (2.12, 16.38)	-0.947	0.344
Treatments during hospitalization					
Ventilator [n (%)]	61 (59.2)	35 (59.3)	26 (59.1)	0.001	0.981
Vasoactive drugs [n (%)]	64 (62.1)	44 (74.6)	20 (45.5)	9.085	0.003 **
Blood transfusion [n (%)]	52 (50.5)	28 (53.8)	24 (54.5)	0.507	0.477
Renal replacement therapy [n (%)]	50 (48.5)	26 (44.1)	24 (54.5)	1.108	0.293

BMI Body mass index, CKD Chronic kidney diseases, APACHE II Acute physiology and chronic health evaluation 2, KDIGO The Kidney Disease: Improving Global Outcomes, Cr Serum creatinine, BUN Blood urea nitrogen, NAGL Neutrophil gelatinase-associated lipocalin, PCT Procalcitonin, Lac Lactic acid, OI Oxygenation index, WBC White blood cell, GR Neutrophil granulocyte, LY Lymphocyte, NLR Neutrophil/ lymphocyte ratio, Th17 Helper T cell 17, Treg, Regulatory T cell, IL-10 Interleukin-10, IL-17 Interleukin-17, TNF-α Tumor necrosis factor alpha, RRT renal replacement therapy. p-value: * 0.05 - 0.01, ** 0.01 - 0.001, * < 0.001.**

without sepsis group, Treg/Th17 ratio in the SAKI group showed a significant increase (0.11 vs. 0.04, $p < 0.001$). Cytokine concentrations in the peripheral blood of patients were also measured. TNF-α is a primary inflammatory factor that is released during the early stages of inflammation. IL-10 and IL-17 are the major cytokines of Treg and Th17 cells. The concentrations of IL-10 and IL-17 were significantly higher in the SAKI group than in the AKI without sepsis group. The concentrations of TNF-α revealed no significant difference between the two groups. Changes in the concentration of IL-17 and the frequency of Th17 cells were consistent. However, changes in IL-10 concentration were not always consistent with the trend in the proportion of Treg cells (see Table 1).

Table 2 showed univariate and multivariate analyses of risk factors for SAKI. Regression analysis was performed with SAKI as the dependent variable and with the factors with significant differences in the univariate analysis as the independent variables. Variables that had a p-value less than 0.05 by the univariable binary logistic regression analysis included age, diabetes, CKD, chronic cardiovascular disease, urine output in 24 hours, Cr, PCT, GR, NLR, the proportion of Treg cells, the proportion of Th17 cells, Th17/Treg ratio, concentration of IL-10, concentration of IL-17, and use of vasoactive drugs were included in the multivariate logistic regression analysis. Independent predictors of SAKI were identified at $p < 0.05$. Multivariate regression analysis revealed that CKD and Th17/Treg ratio were independent risk factors for SAKI (see Figure 3). The AUC demonstrated that the Th17/Treg ratio measured 0.775 (95% CI 0.683 - 0.851, $p < 0.0001$). The cutoff value of Th17/Treg ratio for predicting SAKI was 0.033. When the Th17/Treg ratio was > 0.033, the sensitivity of predicting SAKI was 0.967, and the specificity was 0.500. Then, we divided the AKI patients into high Th17/Treg ratio group (Th17/Treg ratio > 0.033) and low Th17/Treg ratio group (Th17/Treg ratio ≤ 0.033). In the high Th17/Treg ratio group, SAKI prevalence was significantly higher than in the low Th17/Treg ratio group (96.7% vs. 50.0%, $p < 0.001$). Then, univariate and multivariate logistic regression were performed with the occurrence of SAKI as the dependent variable and with

Table 2. Risk factor analysis for SAKI.

	Univariable logistic regression		Multivariable logistic regression	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age	1.031 (1.002 - 1.061)	0.035 *		
Diabetes [n (%)]	2.676 (1.055 - 6.783)	0.038 *		
CKD [n (%)]	0.295 (0.119 - 0.731)	0.008 **	0.184 (0.048 - 0.710)	0.014 *
Chronic cardiovascular disease [n (%)]	0.368 (0.163 - 0.832)	0.016 *		
Urine output - L/24hours (median [Q1, Q2])	0.660 (0.457 - 0.953)	0.027 *		
Cr - µmol/L (median [Q1, Q2])	1.002 (1.000 - 1.004)	0.078		
PCT - ng/mL (median [Q1, Q2])	0.961 (0.933 - 0.991)	0.010 *	0.973 (0.943 - 1.004)	0.085
GR - x 10^9/L (median [Q1, Q2])	0.942 (0.893 - 0.994)	0.029 *		
NLR (median [Q1, Q2])	0.946 (0.907 - 0.986)	0.009 **	0.956 (0.912 - 1.003)	0.068
The proportion of Treg cells - % (median [Q1, Q2])	1.264 (0.994 - 1.609)	0.057		
The proportion of Th17 cells - % (median [Q1, Q2])	0.600 (0.116 - 3.095)	0.542		
Th17/Treg ratio (median [Q1, Q2])	0.010 (0.000 - 0.326)	0.009 **	0.002 (0.000 - 0.172)	0.006 **
IL10 - pg/mL (median [Q1, Q2])	0.996 (0.992 - 1.001)	0.100		
IL17 - pg/mL (median [Q1, Q2])	1.009 (0.986 - 1.033)	0.460		
vasoactive drugs [n (%)]	3.520 (1.529 - 8.105)	0.003 **		

CKD Chronic kidney diseases, Cr Serum creatinine, PCT Procalcitonin, GR Neutrophil granulocyte, LY Lymphocyte, NLR Neutrophil/lymphocyte ratio, Th17 Helper T cell 17, Treg Regulatory T cell, IL-10 Interleukin-10, IL-17 Interleukin-17. p-value: * 0.05 - 0.01, ** 0.01 - 0.001, * < 0.001.**

Table 3. Multivariable logistic regression of reclassification by cutoff value of Th17/Treg ratio.

	Odds ratio (95% CI)	p-value
High Th17/Treg ratio	35.942 (5.199 - 248.484)	0.000 ***
CKD	0.266 (0.079 - 0.892)	0.032 *
PCT	0.970 (0.933 - 1.008)	0.119
NLR	0.937 (0.886 - 0.991)	0.022 *

CKD Chronic kidney diseases, PCT Procalcitonin, NLR Neutrophil/lymphocyte ratio, Th17 Helper T cell 17, Treg Regulatory T cell. p-value: * 0.05 - 0.01, ** 0.01 - 0.001, * < 0.001.**

the binary variable high Th17/Treg ratio as the independent variable (see in Table 3). High Th17/Treg ratio was an independent risk factor for SAKI (OR = 35.942 95% CI 5.199 - 248.484, $p < 0.001$). This result verified the results of the previous regression. Th17/Treg imbalance can be used as an independent risk factor for predicting SAKI in AKI patients.

Acute kidney injury (AKI) and chronic kidney disease are common interconnected syndromes that represent a public health problem [11]. AKI can lead to CKD, and CKD increases the risk of AKI [12]. The above results suggested that CKD and Th17/Treg ratio were independent risk factors for SAKI. Thus, sensitivity analysis done in the subjects after exclusion of patients with history of CKD and had the same conclusion, and Th17/Treg ratio was an independent risk factor for SAKI in AKI patients with different etiology (see Supplementary materials: Table S1 and Table S2).

General outcomes of AKI patients

There were no significant differences in APACHE II score and KDIGO stage between the SAKI group and AKI without sepsis group. Thus, the rate of patients who underwent renal replacement therapy (RRT) was similar (44.1% vs. 54.5%, $p < 0.05$). The proportion of patients in the SAKI group receiving vasoactive drugs

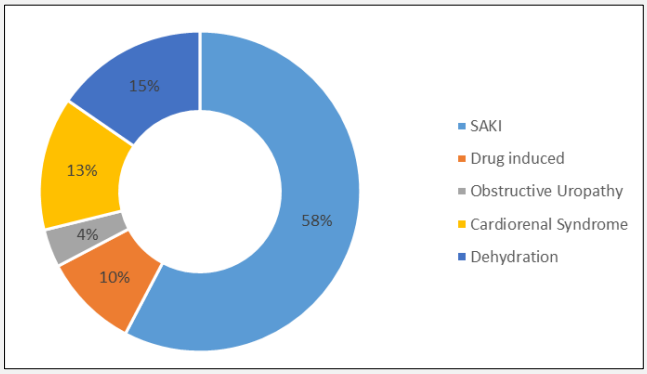

Figure 1. Etiology of AKI in study population, AKI Acute Kidney Injury.

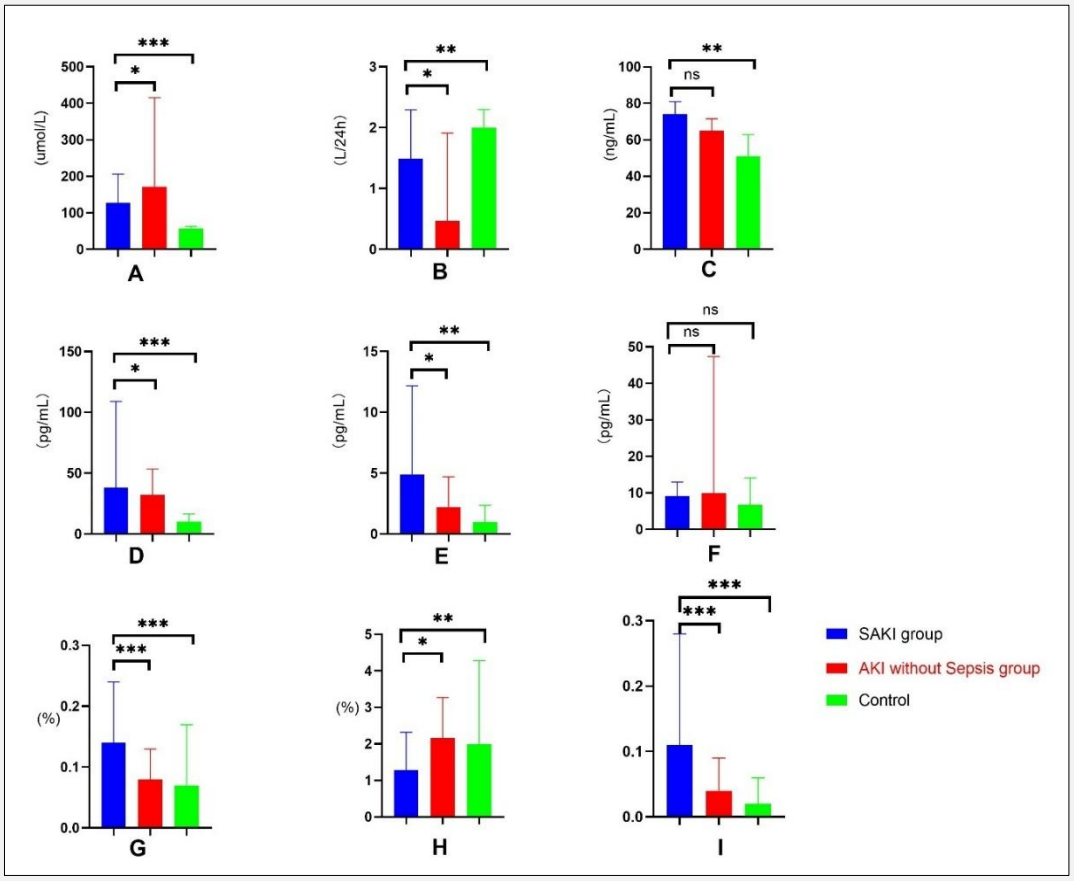

Figure 2. Immune indicators and renal function among SAKI, AKI without sepsis, and healthy volunteers, including the comparison of Cr (A), Urine output (B), NAGL (C), IL-10 (D), IL-17 (E), TNF-α (F), Th17 (G), Treg (H), Th17/Treg ratio (I).

Th17 Helper T cell 17, Treg Regulatory T cell, IL-10 Interleukin-10, IL-17 Interleukin-17, TNF-α Tumor necrosis factor alpha, Cr Serum creatinine, BUN Blood urea nitrogen, NAGL Neutrophil gelatinase-associated lipocalin. p-value: ns > 0.05, * 0.05 - 0.01, ** 0.01 - 0.001, * < 0.001.**

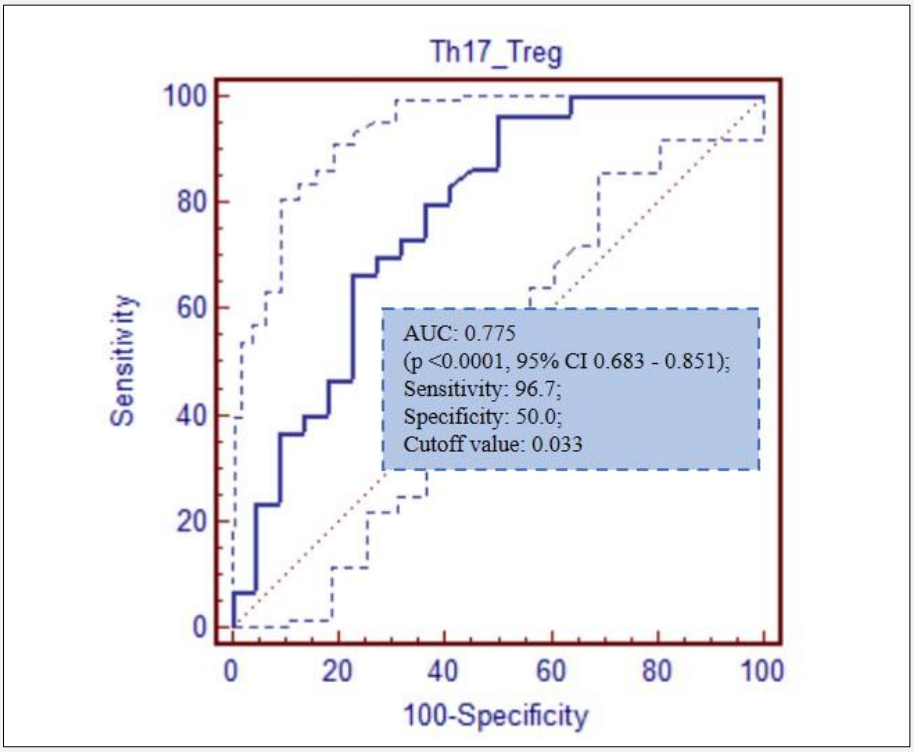

Figure 3. ROC curve of Th17/Treg ratio prediction performance for SAKI.

SAKI Sepsis induced acute kidney injury, ROC receiver operating characteristics, CI confidence interval, Treg Regulatory T cell, Th17 Helper T cell 17.

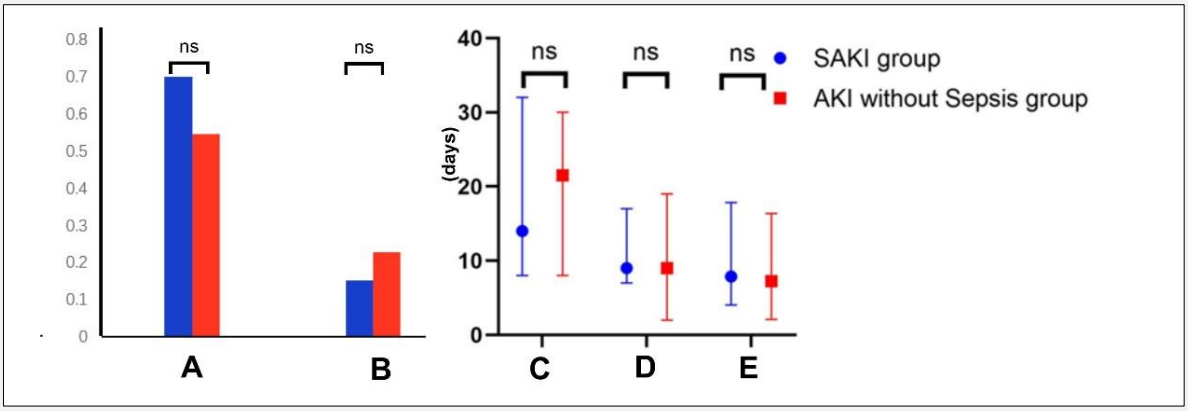

Figure 4. General outcomes between the SAKI group and the AKI without sepsis group, including A the comparison of rate of renal recovery, B the 28-day mortality, C LOS, D ICU length of stay, and E expenses of ICU.

SAKI Sepsis induced acute kidney injury, LOS hospital length of stay, ICU Intensive care unit. p-value: ns > 0.05.

was significantly higher than that in the AKI without sepsis group, which may be related to the primary disease (Table 1).

Overall, there were no notable differences in the study endpoints between the SAKI group and the AKI without sepsis group (Table 1 and Figure 4).

DISCUSSION

Acute kidney injury is an increasingly recognized global public health issue, characterized by high incidence, mortality, and long-term complications, which is due to a variety of causes, such as infection, drugs or hypovolemic shock. The large spectrum of AKI implies diverse pathophysiological mechanisms. Most of our current knowledge is based on animal (mice) models. We endeavored to apply this literature to clinical practice by studying Th17/Treg imbalance in patients with acute kidney injury.

The main finds of our study were that compared with healthy volunteers, there was a Th17/Treg imbalance in AKI patients. Th17/Treg ratio was higher in patients with SAKI than with AKI caused by other causes. For AKI patients, the elevated Th17/Treg ratio was an independent predictor of SAKI. The higher Th17/Treg ratio in patients with SAKI may be related to the specific inflammatory and immune responses characteristic of sepsis. Sepsis often leads to an imbalance in the immune system[13], characterized by a dysregulation between pro-inflammatory and anti-inflammatory responses. In sepsis, the production of pro-inflammatory cytokines (such as IL-17) increases, leading to the activation and proliferation of Th17 cells [6,14,15]. Meanwhile, the function of Treg cells may be inhibited or the numbers reduced, weakening the suppressive immune response [16,17]. This may be due to immune dysregulation triggered by inflammatory mediators and endotoxins, which inhibit the activity of Treg cells. The balance between Th17 cells and Treg cells is disrupted, with an increase in pro-inflammatory Th17 cells and a decrease or dysfunction of Treg cells, resulting in a higher Th17/Treg ratio. Moreover, SAKI is often accompanied by significant changes in the local microenvironment of the kidney [18], including alterations in cytokine levels and immune cell infiltration [19]. These local inflammatory responses may further promote the expansion of Th17 cells and inhibit the function of Treg cells, exacerbating immune imbalance [20,21]. The elevated Th17/Treg ratio in patients with SAKI may result from systemic and local immune imbalances triggered by sepsis, reflecting the dysregulation between pro-inflammatory and anti-inflammatory systems in sepsis [6].

Although SAKI patients had a higher Th17/Treg ratio, the rate of renal recovery and 28-day mortality were not significantly different compared to AKI caused by other etiologies. This can be explained from the following perspectives. First, different immune mechanisms affect renal recovery in different ways. AKI caused by different etiologies presents varying immune responses, which may affect the mode of renal function recovery. The immune response in SAKI is complex and variable [22,23], potentially leading to more severe initial damage. However, with appropriate anti-infective treatments and supportive care, renal function may still recover. Other types of AKI (such as drug toxicity or ischemia-reperfusion injury) may have different early injury mechanisms, but recovery mechanisms also vary [24-28]. Therefore, both groups of patients show similar recovery rates in renal function. Second, Th17/Treg ratio is not the only factor determining prognosis although Th17/Treg ratio reflects the balance between pro-inflammatory and anti-inflammatory responses of the immune system [29]. The prognosis of patients with SAKI is influenced by a variety of complex factors. These include the underlying condition, the degree of damage to other organs, treatment measures, etc. Impact of therapeutic interventions in patients with SAKI, including the use of antibiotics, infection source control, anti-inflammatory treatments, and renal replacement therapies, may have effectively reduced the negative impact of a high Th17/Treg ratio [30]. The Th17/Treg ratio is just one indicator of immune imbalance, but it does not solely determine prognosis or mortality. Other factors such as hemodynamic status, infection control, and the body's compensatory capacity also play important roles. Finally, the elevation of the Th17/Treg ratio may be an adaptive response to sepsis [31]. In sepsis, the immune system is hyperactivated to fight infection, leading to an increase in Th17 cells and suppression of Treg cell function. An elevated Th17/Treg ratio does not necessarily lead to a worse prognosis and may help control infection and inflammation in some cases to maintain a relatively stable survival rate and renal function recovery rate. In summary, SAKI patients have a higher Th17/Treg ratio, indicating a stronger pro-inflammatory response, which does not necessarily imply a worse clinical outcome. Multiple factors may contribute to the lack of significant differences in mortality and renal function recovery rates between septic and non-septic AKI patients. Changes in a single immunological indicator cannot fully predict clinical outcomes in complex conditions. More powerful indicators need to be explored in the future to predict the prognosis of AKI. There were several limitations of the study. First, the small sample size and the single-center study design limited the generalizability and external validity of the results. The present data suggested the correlation between Th17/Treg imbalance and AKI patients which may lead the research interests. We have made some improvements in data analysis, such as using the Bootstrap method in logistic regression to make up for the limitations of small samples. Second, this was a correlational study and did not investigate the biological mechanisms involved. We reviewed the related literature to study the potential mechanisms. Further preclinical studies are needed to determine precisely the mechanism involved. Finally, we collected blood samples

from patients instead of tissue specimens. Actually, obtaining tissue samples from critically ill patients carries risks, making it difficult to achieve. The results of blood samples confirmed that it was safe and feasible to analyze the correlation with AKI and Th17/Treg imbalance in peripheral blood samples.

CONCLUSION

There is an imbalance of Th17/Treg in acute kidney injury. Compared with AKI caused by other factors, Th17/Treg ratio is higher in patients with SAKI. However, the 28-day mortality and renal function recovery rate between the two groups of patients do not differ.

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Ethics Approval and Consent to Participate:
This study was approved by the Medical Ethics Committee of Beijing Friendship Hospital, Capital Medical University (No. 2021-P2-006-02).

Consent for Publication:
Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Availability of Data and Materials:
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Interest:
The authors declare that they have no competing interests.

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