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Posted Date: 20 November 2023

doi: 10.20944/preprints202311.1224.v1

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Association of WBC levels with All-Cause Mortality in ICU Patients Receiving CRRT: A Retrospective Cohort Study

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Abstract: Background: Acute kidney injury (AKI) is a common comorbidity in the intensive care unit (ICU), with higher mortality rate among those who receive continuous renal replacement therapy (CRRT). This study aimed to investigate the association of white blood cell (WBC) levels with all-cause mortality in AKI patients undergoing CRRT. **Method:** We conducted a post hoc analysis of ICU patient data from multiple centers in Korea. First, the levels of WBCs were classified into four groups based on quartiles. Second, the relationship between different WBC levels and mortality was investigated with the Kaplan-Meier (K-M) survival curves, Cox proportional hazards model, and generalized additivity model (GAM). Finally, we used multiple imputation to avoid bias caused by non-randomized missing data. The endpoints were short-term (28 days) and long-term (90 days) mortality. **Result:** In our analysis, a total of 1,100 ICU patients were included. Among them, 670 (60.90%) and 781 (71.00%) died within 28 and 90 days, respectively. K-M survival curves showed that patients with the lowest WBC levels had the highest risk of mortality for both outcomes. Adjusted multivariate Cox regression analysis confirmed that compared with the reference level (quartile 2), low WBC levels increased the risk of both 28-day mortality (quartile 1, HR = 1.43, 95% CI: 1.13–1.83, P = 0.004) and 90-day mortality (quartile 1: HR = 1.32; 95% CI: 1.05-1.66, P = 0.016). GAM analysis showed a non-linear, U-shaped relationship between pre-CRRT WBC levels and all-cause mortality (non-linear $P < 0.05$). **Conclusion:** The baseline WBC level is an independent predictor of prognosis for ICU patients receiving CRRT. Patients in the lowest WBC quartile had the highest risk of all-cause mortality. These results provide further evidence that early observation and intervention may have a prognostic value for these high-risk patients.

Keywords: acute kidney injury; continuous renal replacement therapy; intensive care unit; cox regression; prediction model

Introduction

Acute kidney injury (AKI) is an organ dysfunction syndrome with adverse clinical outcomes in intensive care unit (ICU) setting. Over 50% of patients in ICU develop AKI at some point in their ICU stay [1]. As renal function deteriorates, the risk of mortality in patients increases [2]. Therefore, early identification and intervention may improve the prognosis of AKI patients [3]. In clinical practice, the prognosis of AKI patients is often evaluated based on changes in serum creatinine and urine output. However, these indicators are not very sensitive and by the time they vary, significant kidney injury has already occurred. Therefore, the search of other biomarkers for early AKI detection has been a research priority to guide clinical treatment decisions.

AKI is a common diagnosis among patients in the ICU, typically caused by massive fluid loss or sepsis, which can increase the risk of mortality [4,5]. Continuous renal replacement therapy (CRRT) is the recommended treatment for ICU patients requiring blood filtration [6]. Compared with

 doi:10.20944/preprints202311.1224.v1

traditional renal replacement therapy, CRRT has been demonstrated to offer superior hemodynamic tolerance and improved survival rates [7,8]. Despite the provision of intensive dialysis care, the risk of mortality is still high among patients with severe AKI who require CRRT [9]. Therefore, early identification of the mortality risk of these critically ill patients is of great significance not only to make the best use of medical resources but also to reduce the burden on healthcare professionals. Although some biomarkers can detect early changes in kidney function that are associated with AKI, they are less sensitive in predicting the prognosis of patients [10]. Currently, the most widely used methods for assessing critically ill patients include the Sequential Organ Failure Assessment (SOFA) score and Acute Physiology and Chronic Health Evaluation II (APACHE II) score, which are more specific and allow for comprehensive evaluation of patients [11,12]. However, none of these indexes are accurate predictors of all-cause mortality [13,14] .

White blood cell (WBC) is an essential component of the human immune system that is responsible for protection against foreign pathogens and substances [15]. It is useful as an indicator of the immune status and for the clinical diagnosis of infectious conditions. Studies have also shown an independent correlation between WBC counts and the incidence of organ injury [16–18]. Further studies have demonstrated that the degree of WBC infiltration in the kidneys is associated with the prognosis of AKI [19–21]. In addition, reducing WBC infiltration in renal tubules and interstitium can improve kidney injury to some extent [22,23]. Although the role of WBC infiltration in AKI is well established in research, the clinical significance of WBC count remains uncertain. In particular, whether WBC count can predict mortality in critically ill AKI patients receiving CRRT remains unclear.

Because the mortality rate among AKI patients in ICU is high, early identification poor prognosis can improve the treatment effects and prognosis of patients. The detection of WBC has broad applications in clinical practice, and previous studies have revealed that WBC counts can provide some prognostic information for AKI. In this study, using data from a multicenter study of 2391 ICU patients, we investigated (i) the mortality rates and clinical characteristics of critically ill AKI patients with different WBC levels and (ii) the impact of other risk factors on this relationship. Our findings provide insights into the potential clinical value of WBC counts in identifying patients with a high mortality risk and guiding optimal allocation of ICU resources.

Method

Study population

This post-hoc analysis was conducted using retrospective data obtained from the Dryad database. The data had been uploaded and collected by Sun et al. from two hospitals: Yonsei University Health System Severance Hospital and National Health Insurance Service Medical Center Ilsan Hospital [24]. In brief, the previous study included 2391 ICU patients with AKI who met the AKIN criteria (i.e., twofold increase in creatinine and urine output <0.5 ml/kg.h) and underwent CRRT between 2009 and 2016. The data were partially censored based on the following exclusion criteria: (1) stage I AKIN; (2) age ≤18 years; (3) pregnancy or lactation; (4) prior history of chronic kidney disease or receipt of CRRT; (5) post-renal obstruction; and (6) prior renal transplantation. In addition, we excluded participants with extreme WBC count values (<100/μl or >90000/μl) and those without survival time data. Ultimately, data from 1100 patients who met the inclusion criteria were analyzed in this study (Figure 1). Since the previous study was approved by the Yonsei University Health System Severance Hospital Institutional Review Board, further ethical approval was not required for this study.

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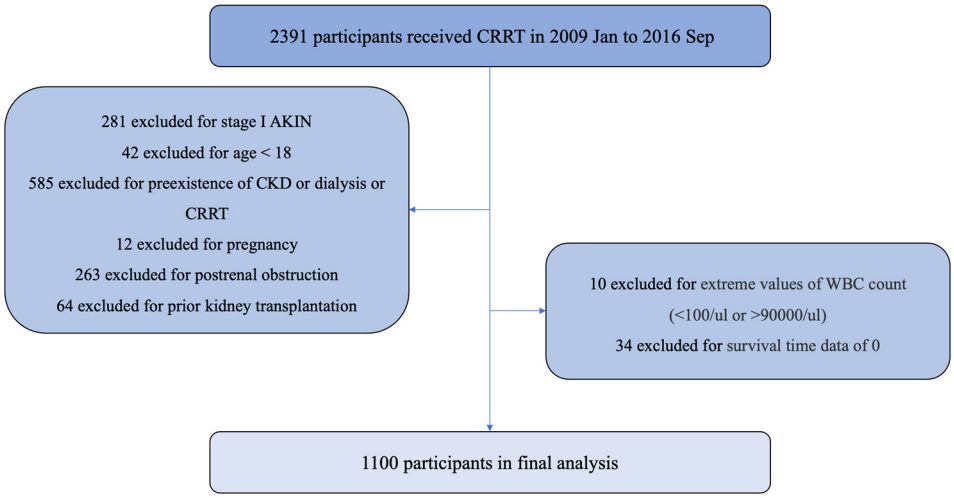

Figure 1. Flowchart of study population screen *CRRT*: Continuous renal replacement therapy, *AKIN*: Acute Kidney Injury Network, *CKD*: Chronic kidney disease, *WBC*: White blood cell.

Data collection

Patient-level clinical data were captured using standardized spreadsheets, including age, sex, weight, blood pressure, and comorbidities. In addition, biochemical laboratory data, including hemoglobin (Hb), white blood cell (WBC) count, blood creatinine (Cr), albumin (Alb), bicarbonate (HCO3-), potassium ion (K+), lactate, alanine transaminase (ALT), aspartate aminotransferase (AST), and total bilirubin, were collected both before and after initiation of CRRT. Clinical scores, such as the glomerular filtration rate (GFR), Charlson comorbidity index (CCI), Sequential Organ Failure Assessment (SOFA) score, and Acute Physiology and Chronic Health Evaluation II (APACHE II) score, were also recorded. All characteristics were stratified into quartiles based on WBC counts. The endpoints were 28-day and 90-day mortality rates among patients who underwent CRRT.

CRRT protocol

Nephrologists perform regular evaluation of the development course of AKI in ICU patients to determine whether to initiate CRRT for critically ill patients. Initiation of CRRT is generally indicated when sustained oliguria, severe metabolic acidosis, volume overload, and uncontrolled hyperkalemia are detected. Common access sites for initiating hemodiafiltration include the internal jugular, subclavian, and femoral veins. The initial blood flow rate for CRRT is typically set at 100 ml/min, with subsequent increases to 150 ml/min as tolerated. Clinical guidelines recommend targeted dialysis volume and delivery of optimal replacement dose to manage patients with AKI.

Statistical analysis

Patients were classified into four groups (Quartile 1, 2, 3, and 4) based on the different levels of WBC counts. Continuous variables with normal distribution were expressed as mean ± standard deviation and compared using independent t-test. Continuous variables with skewed distribution were expressed as interquartile range and compared using the Wilcoxon signed rank test. The Kaplan-Meier (K-M) estimator to calculate cumulative survival proportions for patients with varying WBC levels. The resulting survival curves were then analyzed using the log-rank method to identify any statistically significant differences between them. Cox proportional hazard models were constructed based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. Three models were analyzed to investigate the independent association between WBC levels and all-cause mortality in CRRT patients. The crude model was unadjusted, but in model I demographic variables were adjusted. In model II, which was based on model I, variables

 doi:10.20944/preprints202311.1224.v1

with p-value less than 0.05 in univariate cox regression were adjusted for the two endpoint events. The results were expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). After incorporating the median of each WBC group into the Cox model, we performed a trend test to analyze whether there was a linear trend between WBC and mortality. In addition, we used the generalized additive model (GAM) to identify nonlinear relationships and performed a likelihood ratio test.

The missing pattern of some variables could not be determined to be missing not at random (MNAR) in this study. In addition, because arbitrary analyses may have led to bias, we used multiple imputation to assess the impact of missing data. Five sets of imputed data were generated based on chain equations, and the variables involved were included. Cox regression analyses were sequentially performed with the five data sets and their HR and 95% CI were combined using Rubin's Rule [25]. If the difference between the combined HR and the original HR was less than 10%, the effect of the missing data on the regression model was not considered significant. The adjusted variables of the stratified analysis were consistent with model II, but the stratified variables were not involved in the adjustment. The interaction was validated using likelihood ratio test to analyze whether the effect of WBC on mortality was significantly different between stratification levels. All analyses were performed using R (version 4.1.2), and a P-value of less than 0.05 (two-tailed) was considered statistically significant.

Result

Baseline characteristics of Participants

In total, 1100 AKI patients who received CRRT in ICU were included in this study. The mean age of the patients was 63.50 ± 14.20 years, and 673 (61.18%) patients were male. The mortality was 670 (60.90%) and 781 (71.00%) within 28 days and 90 days, respectively. Baseline WBC levels did not follow a normal distribution ($P < 0.05$), and were categorized into four groups based on quartiles. The WBC count range were 100-6680, 6680-11725, 11725-18710, and 18710-68400 in Quartile 1, Quartile 2, Quartile 3, and Quartile 4, respectively. At baseline (Table 1), compared with patients with high WBC counts, patients with low WBC counts had higher levels of bicarbonate, GFR and SOFA scores. In contrast, patients with high WBC counts at baseline were older, had elevated levels of serum potassium, hemoglobin, creatinine, and albumin, and had higher rates of complications, such as hypertension, dementia and congestive heart failure ($P < 0.05$).

Table 1. Baseline characteristics of all participants according to WBC quartiles.

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	Q1 [100,6680]	Q2 (6680,11725]	Q3 (11725,18710]	Q4 (18710,68400]	p.trend
	N=276	N=274	N=276	N=274	
Age (years)	60.94 (13.39)	64.31 (13.87)	64.03 (15.41)	64.73 (13.80)	0.004
Sex:					0.368
Male	167 (60.51%)	183 (66.79%)	160 (57.97%)	163 (59.49%)	
Female	109 (39.49%)	91 (33.21%)	116 (42.03%)	111 (40.51%)	
Myocardial infarction (%)	12 (4.35%)	30 (10.95%)	46 (16.67%)	21 (7.66%)	0.050
Congestive heart failure (%)	22 (7.97%)	51 (18.61%)	64 (23.19%)	44 (16.06%)	0.004
Cerebrovascular disease (%)	17 (6.16%)	34 (12.45%)	29 (10.55%)	29 (10.66%)	0.149
Peripheral vascular disease (%)	6 (2.17%)	12 (4.38%)	14 (5.07%)	12 (4.38%)	0.166
Dementia (%)	5 (1.81%)	13 (4.74%)	8 (2.90%)	16 (5.84%)	0.048
Diabetes mellitus (%)	81 (29.35%)	100 (36.50%)	110 (39.86%)	94 (34.43%)	0.146
Hypertension (%)	133 (48.19%)	143 (52.19%)	148 (53.62%)	159 (58.03%)	0.022
COPD (%)	22 (7.97%)	19 (6.93%)	15 (5.43%)	22 (8.03%)	0.845
Potassium (mEq/L)	4.30 [3.90;4.90]	4.50 [3.90;5.30]	4.70 [4.05;5.40]	4.60 [4.10;5.30]	0.001
Bicarbonate (mEq/L)	17.00 [13.00;21.00]	17.00 [13.00;21.75]	17.00 [13.75;21.00]	16.00 [12.00;20.00]	0.047
Phosphate (mEq/L)	5.30 [4.00;6.77]	5.30 [3.90;6.75]	5.60 [4.20;7.10]	5.60 [4.03;7.50]	0.061
CCI	3.00 [2.00;5.00]	3.00 [2.00;4.00]	3.00 [1.75;5.00]	3.00 [2.00;5.00]	0.898
BMI (kg/m2)	23.10 [20.49;25.88]	23.99 [21.76;26.34]	23.68 [20.77;26.02]	23.84 [21.15;26.69]	0.276
MAP (mmHg)	77.85 [68.53;86.30]	76.85 [69.70;85.60]	76.70 [67.00;86.47]	75.30 [66.00;88.00]	0.176
MV (%)	221 (80.36%)	224 (81.75%)	210 (76.09%)	206 (75.18%)	0.057
Hemoglobin (g/dL)	8.90 [8.00;10.15]	9.40 [8.50;10.80]	9.40 [8.30;10.70]	9.60 [8.50;11.30]	<0.001
BUN (mg/dl)	47.00 [33.00;71.00]	49.50 [34.00;74.00]	52.00 [33.00;78.00]	48.00 [33.00;68.00]	0.733
Creatinine (mg/dL)	2.28 [1.67;3.07]	2.38 [1.62;3.42]	2.44 [1.62;3.60]	2.53 [1.75;3.69]	0.003
Albumin (g/dL)	2.50 [2.12;2.80]	2.70 [2.30;3.10]	2.70 [2.40;3.10]	2.60 [2.20;2.90]	0.018
CRP (mg/L)	87.65 [28.17;209.02]	66.81 [16.94;155.60]	67.50 [19.30;149.00]	94.10 [25.55;161.95]	0.511
GFR (mL/min1.73m2)	27.20 [19.20;39.30]	26.75 [16.38;40.02]	25.90 [16.50;38.55]	24.80 [15.10;34.50]	0.003
UO (mL)	30.00 [5.00;90.00]	40.00 [10.00;110.00]	30.00 [0.00;97.50]	35.00 [5.00;90.00]	0.937
APACHE II score	28.00 [23.00;33.00]	27.00 [21.00;32.00]	27.00 [21.50;32.00]	28.00 [22.00;33.00]	0.502
SOFA score	13.00 [11.00;15.00]	12.00 [9.00;14.00]	11.00 [9.00;14.00]	12.00 [10.00;14.00]	0.001
Cause of CRRT:					0.402
Volume overload	32 (11.59%)	48 (17.52%)	40 (14.49%)	29 (10.58%)	
Metablic acidosis	66 (23.91%)	58 (21.17%)	48 (17.39%)	64 (23.36%)	
Hyperkalemia	12 (4.35%)	16 (5.84%)	14 (5.07%)	10 (3.65%)	
Uremia	22 (7.97%)	29 (10.58%)	31 (11.23%)	29 (10.58%)	
Oliguria	73 (26.45%)	69 (25.18%)	71 (25.72%)	74 (27.01%)	
Others	71 (25.72%)	54 (19.71%)	72 (26.09%)	68 (24.82%)	
CRRT dose (mL/kg*h)	37.08 [33.82;39.93]	36.41 [33.71;39.22]	36.79 [34.19;39.30]	36.50 [34.39;39.52]	0.776
AKIN stages (%):					0.255
Stage 2	78 (28.26%)	73 (26.64%)	65 (23.55%)	68 (24.82%)	
Stage 3	198 (71.74%)	201 (73.36%)	211 (76.45%)	206 (75.18%)	
Cause of AKI:					0.376
Sepsis	198 (71.74%)	206 (75.18%)	176 (63.77%)	197 (71.90%)	
Nephrotoxic	12 (4.35%)	5 (1.82%)	6 (2.17%)	12 (4.38%)	
Ischemia	22 (7.97%)	23 (8.39%)	22 (7.97%)	22 (8.03%)	
Surgery	17 (6.16%)	21 (7.66%)	29 (10.51%)	23 (8.39%)	
Others	27 (9.78%)	19 (6.93%)	43 (15.58%)	20 (7.30%)	
28 days mortality	208 (75.36%)	153 (55.84%)	156 (56.52%)	153 (55.84%)	<0.001
90 days mortality	233 (84.42%)	181 (66.06%)	184 (66.67%)	183 (66.79%)	<0.001

Continuous measures for normal distribution are expressed as mean ± SD, continuous measures for nonnormal distribution are expressed as interquartile range (IQR), and counts are expressed as n (%). Continuous variables were compared between groups using ANOVA and Kruskal–Wallis analysis, with the chi-square test used for count data. *COPD*: chronic kidney disease, *CCI*: Charlson Comorbidity Index, *BMI*: body mass index, *MAP*: mean arterial pressure, *MV*: mechanical ventilation, *BUN*: blood urea nitrogen, *CRP*: C-reactive protein, *GFR*: glomerular filtration rate, *UO*: urine output, *APACHE II*: Acute Physiology and Chronic Health Evaluation II, *SOFA*: Sequential Organ Failure Assessment.

Relationship between amount of WBC and all-cause mortality

K-M curve analysis demonstrated that patients in the lowest quartile of WBC levels (Quartile 1) had significantly higher long-term mortality rates than those in the higher WBC groups (Quartiles 2, 3, or 4) in two endpoint events (log-rank analysis; $P < 0.01$; Figure 2). The results of univariate Cox regression analysis revealed that 15 variables were significantly associated with all-cause mortality during both the 28-days and 90-days follow-up periods, including WBC counts, SOFA score, and APA II score (Supplementary Table 1). Therefore, we included these variables in the final multivariate Cox regression (Model II) along with demographic variables to correct for bias caused by the effect of these variables on mortality due to WBC counts.

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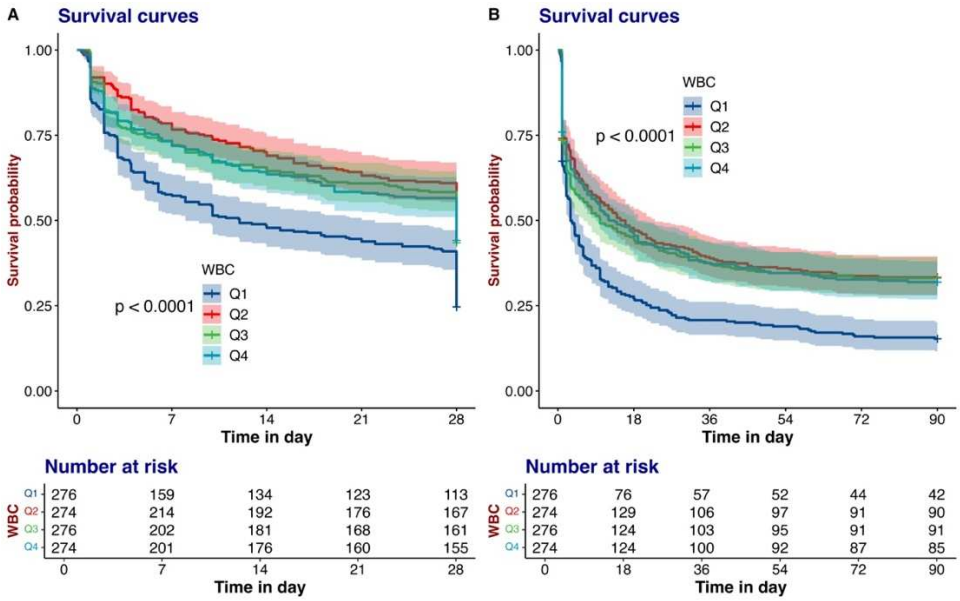

Figure 2. Kaplan–Meier survival curve of all-cause mortality based on quartiles of white blood cell (WBC) counts. A.28-days mortality, B.90-days mortality.

To assess the relationship between WBC levels and all-cause mortality, we chose the second quartile of the WBC range as the reference category. For 28-days mortality, the crude model showed that compared with the reference category, the HR (95% CI) of the first quartile was 1.78 (1.44-2.19; P < 0.001). When adjusted for age and gender in model 1, a similar trend was observed, with the HR (95% CI) of the first quartile of 1.82 (1.47-2.25; P < 0.001). With further adjustment for possible confounders in model II, patients in the other WBC levels showed a significantly higher risk of long-term mortality than reference groups (Compared with Quartile 2, aHR = 1.43, 95% CI: 1.13–1.83, P = 0.004 for Quartile 1; aHR = 1.10, 95% CI: 0.85–1.41, P = 0.472 for Quartile 3; and aHR = 1.04, 95% CI: 0.81–1.35, P = 0.756 for Quartile 4) (Table 2). In Table 2, the unadjusted model, the model I adjusted for demographic characteristics, and the fully adjusted model II are presented. It shows a similar trend in 90-day mortality, especially for model II (Q1: HR = 1.32; 95% CI: 1.05-1.66, P = 0.016; Q2: HR = 1 (reference); Q3: HR = 1.08; 95% CI: 0.85-1.36 P = 0.543; Q4: HR = 0.97; 95% CI: 0.77-1.23 P = 0.790). The Cox models met the proportional hazards assumption, as determined using the Schoenfeld residual test (P > 0.05).

Table 2. Cox regression analysis of WBC versus all-cause mortality in short-term (28 days) and long-term (90 days).

 doi:10.20944/preprints202311.1224.v1

	Crude Model HR (95% CI)	Model I HR (95% CI)	Model II HR (95% CI)
28 days death			
No. of events	670	670	538
Q1	1.78 [1.44;2.19] < 0.001	1.82 [1.47;2.24] < 0.001	1.43 [1.13;1.83] 0.004
Q2	Ref.	Ref.	Ref.
Q3	1.06 [0.85;1.33] 0.601	1.07 [0.86;1.34] 0.527	1.10 [0.85;1.41] 0.472
Q4	1.06 [0.84;1.32] 0.626	1.07 [0.85;1.33] 0.576	1.04 [0.81;1.35] 0.756
90 days death			
No. of events	781	781	622
Q1	1.68 [1.38;2.04] < 0.001	1.72 [1.41;2.09] < 0.001	1.32 [1.05;1.66] 0.016
Q2	Ref.	Ref.	Ref.
Q3	1.05 [0.86;1.29] 0.632	1.06 [0.86;1.30] 0.574	1.08 [0.85;1.36] 0.543
Q4	1.04 [0.85;1.28] 0.720	1.04 [0.85;1.28] 0.689	0.97 [0.77;1.23] 0.790

Crude model: unadjusted; Model I: adjusted for age and sex; Model II: adjusted for age, sex, DM, HTN, HCO3-, PO43-, CCI, MAP, MV, Hb, Cre, Alb, UO, APACHE II score, SOFA score, and Cause of CRRT. *DM*: diabetes mellitus, *HTN*: hypertension, HCO_3^-: bicarbonate, PO_4^{3-}: phosphate, *Hb*: hemoglobin, *Cre*: creatinine, *Alb*: albumin, *HR*: hazard ratio, *CI*: confidence interval.

Results of non-linearity in WBC levels and all-cause mortality

We performed GAM analysis to further investigate the nonlinear relationship between blood WBC levels and all-cause mortality at 28 and 90 days (Figure 3). The results of the Cox regression model using smoothed curves and proportional hazards using cubic spline functions showed a non-linear association between the number of WBCs and all-cause mortality in the adjusted model II (nonlinear $P < 0.001$). In the 28-day follow-up, we selected two points where HRs were equal 1 (11000 and 25000 μL) as cutoff values. The mortality of the participants was lowest when the WBC counts was in between the two values. Accordingly, AKI patients with CRRT were divided into very high (< 11000/μL), low (11000 ≤ WBC ≤ 25000/μL), and high (> 25000/μL) risk of mortality groups, respectively (Figure 2A). The same trend was found at the 90-day follow-up. Based on the U-shaped Cox proportional hazards regression model with cubic spline functions. WBC counts were classified into three groups: < 11000, 11000-27000, and ≥ 27000/μL (Figure 2B). These results revealed a U-shaped association between WBC levels and all-cause mortality at both 28-day and 90-day follow-up, with both low and high levels of WBC associated with an increased risk of mortality.

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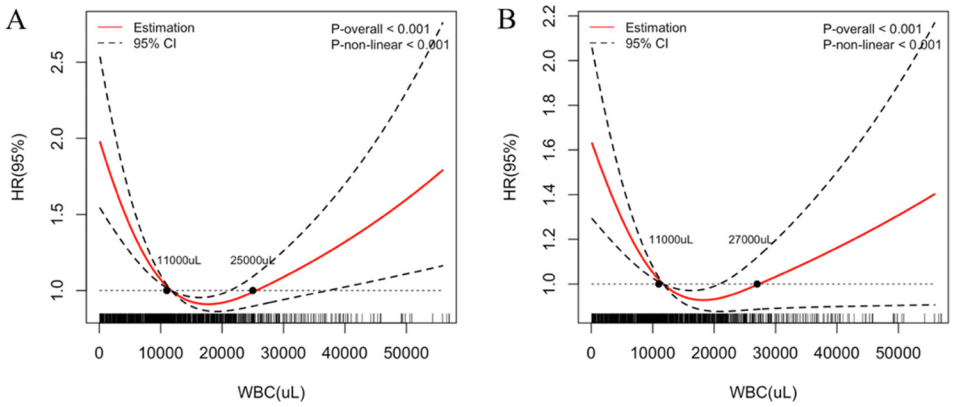

Figure 3. Restricted cubic spline (RCS) plots of adjusted dose–response relationships for WBC counts and all-cause mortality, with density plots indicating the distribution of WBC. A.28-days mortality, B.90-days mortality. All models were adjusted for age, sex, DM, HTN, HCO3-, PO43-, CCI, MAP, MV, Hb, Cre, Alb, UO, APACHE II score, SOFA score, Cause of CRRT.

Multiple imputation

Missing data were imputed for several variables, including cerebrovascular disease, diabetes, potassium, bicarbonate, phosphate, creatinine, BMI, SBP, DBP, mitral valve disease, WBC, hemoglobin, BUN, albumin, CRP, GFR, UO, SOFA score, and APA II score. Missing values for each of these variables were 4, 1, 12, 128, 74, 6, 23, 2, 2, 1, 10, 4, 6, 10, 274, 9, 6, 3, and 13, respectively. The difference between the HR of the Cox regression analysis combined with the 5 sets of imputation data and the HR of the original Cox regression was less than 10% (Supplementary Figure 1) [26]. This indicated that the missing data were missing at random and were unlikely to significantly affect the results of the Cox regression analysis.

Subgroup analysis

We further assessed whether the association between WBC and all-cause mortality could be explained by patients' demographic data and comprehensive clinical score. We conducted multivariable-adjusted hazard ratios for all-cause mortality stratified by age, gender, CCI, APACHE II score, SOFA score and AKI cause using the likelihood ratio test. The results of the subgroup analyses and tests for the interaction of the correlations between WBC levels and mortality at 28 days and 90 days are presented in Table 3. The relationship between white blood cell (WBC) levels and the endpoints remained consistent across almost all subgroups, indicating a stable correlation. The interaction analysis revealed that CCI played an interactive role in the association between WBC levels and mortality at both 28 and 90 days (P for interaction = 0.018 and 0.015 for mortality at 28 days 90 days, respectively). Among patients with lowest WBC levels. those with CCI > 2 had higher HRs (HR = 1.62, 95% CI: 1.11–2.37; HR = 1.39, 95% CI: 0.97–2.00) than those with CCI ≤ 2 (HR = 1.29, 95% CI: 0.88–1.89, HR = 1.08, 95% CI: 0.76–1.55) for both 28-day and 90-day follow-ups. Besides, male patients with first quartile WBC counts (HR = 1.46, 95% CI: 1.08–1.98; P for interaction = 0.014) had a higher risk of mortality within 28 days. Other subgroups did not exhibit statistically significant correlations.

Table 3. The relationship between WBC counts and all-cause mortality of different subgroups in short-term (28 days) and long-term (90 days).

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	No. of Event	Q1	Q2	Q3	Q4	p.interaction
28 days death						
Age						0.933
< 55	127	1.37 [0.84; 2.23]	ref	0.55 [0.31; 0.98]	0.98 [0.55; 1.75]	
>= 55	411	1.48 [1.11; 1.97]	ref	1.37 [1.03; 1.83]	1.10 [0.82; 1.48]	
Sex						0.014
Male	334	1.46 [1.08; 1.98]	ref	1.14 [0.82; 1.58]	1.27 [0.92; 1.74]	
Female	204	1.42 [0.92; 2.17]	ref	1.02 [0.68; 1.53]	0.76 [0.48; 1.18]	
CCI						0.018
<= 2	218	1.29 [0.88; 1.89]	ref	0.98 [0.65; 1.46]	0.83 [0.55; 1.24]	
> 2	226	1.62 [1.11; 2.37]	ref	1.07 [0.71; 1.60]	1.39 [0.92; 2.08]	
APACHE II score						0.135
<= 20	72	1.08 [0.54; 2.15]	ref	0.61 [0.28; 1.35]	1.06 [0.53; 2.13]	
> 20 <= 29	221	1.28 [0.87; 1.89]	ref	0.98 [0.66; 1.47]	0.70 [0.45; 1.09]	
> 29	245	1.92 [1.33; 2.78]	ref	1.34 [0.90; 2.00]	1.44 [0.99; 2.11]	
SOFA score						0.372
<= 11	121	2.11 [1.24; 3.59]	ref	1.27 [0.77; 2.10]	0.75 [0.42; 1.34]	
> 11 <= 13	191	1.29 [0.83; 1.98]	ref	0.87 [0.56; 1.35]	1.25 [0.82; 1.91]	
> 13	226	1.56 [1.08; 2.26]	ref	1.24 [0.80; 1.90]	1.16 [0.76; 1.76]	
AKI cause						0.696
Volume overload	58	2.12 [0.96; 4.69]	ref	1.65 [0.76; 3.56]	0.82 [0.36; 1.86]	
Metablic acidosis	128	1.09 [0.63; 1.90]	ref	0.85 [0.47; 1.53]	1.02 [0.60; 1.72]	
Hyperkalemia	28	6.60 [0.95; 45.81]	ref	10.32 [2.12; 50.29]	1.90 [0.41; 8.85]	
Uremia	45	3.03 [1.06; 8.63]	ref	1.15 [0.49; 2.71]	0.53 [0.18; 1.58]	
Oliguria	132	1.44 [0.88; 2.36]	ref	0.76 [0.45; 1.30]	0.98 [0.57; 1.66]	
Others	147	1.54 [0.91; 2.61]	ref	1.46 [0.85; 2.49]	1.04 [0.59; 1.82]	
90 days death						
Age						0.650
< 55	149	1.36 [0.85; 2.18]	ref	0.65 [0.39; 1.08]	0.86 [0.50; 1.47]	
>= 55	473	1.30 [1.00; 1.70]	ref	1.28 [0.98; 1.68]	1.00 [0.77; 1.31]	
Sex						0.244
Male	383	1.44 [1.09; 1.92]	ref	1.19 [0.88; 1.61]	1.04 [0.77; 1.41]	
Female	239	1.12 [0.76; 1.66]	ref	0.92 [0.63; 1.35]	0.82 [0.56; 1.22]	
CCI						0.015
<= 2	256	1.08 [0.76; 1.55]	ref	0.83 [0.57; 1.20]	0.76 [0.53; 1.09]	
> 2	251	1.39 [0.97; 2.00]	ref	1.17 [0.80; 1.71]	1.29 [0.88; 1.90]	
APACHE II score						0.264
<= 20	85	1.27 [0.65; 2.46]	ref	1.00 [0.49; 2.02]	1.19 [0.63; 2.23]	
> 20 <= 29	268	1.35 [0.95; 1.91]	ref	1.05 [0.74; 1.50]	0.74 [0.51; 1.09]	
> 29	269	1.38 [0.96; 1.98]	ref	1.07 [0.73; 1.57]	1.20 [0.83; 1.73]	
SOFA score						0.529
<= 11	157	1.63 [1.01; 2.63]	ref	1.30 [0.83; 2.03]	0.90 [0.55; 1.45]	
> 11 <= 13	213	1.43 [0.96; 2.15]	ref	0.98 [0.65; 1.47]	1.01 [0.68; 1.51]	
> 13	252	1.16 [0.82; 1.66]	ref	0.97 [0.64; 1.47]	0.95 [0.64; 1.41]	
AKI cause						0.862
Volume overload	72	1.13 [0.55; 2.28]	ref	1.02 [0.51; 2.05]	0.52 [0.25; 1.10]	
Metablic acidosis	139	1.17 [0.71; 1.95]	ref	1.34 [0.75; 2.39]	1.27 [0.77; 2.10]	
Hyperkalemia	34	3.04 [0.71; 13.02]	ref	1.80 [0.49; 6.69]	1.47 [0.36; 6.07]	
Uremia	56	1.15 [0.46; 2.86]	ref	0.54 [0.24; 1.23]	0.77 [0.29; 2.04]	
Oliguria	154	1.64 [1.03; 2.61]	ref	0.78 [0.48; 1.27]	0.77 [0.47; 1.26]	
Others	167	1.45 [0.88; 2.38]	ref	1.41 [0.86; 2.33]	1.26 [0.74; 2.13]	

Except for its stratification variables, all models were adjusted for age, sex, DM, HTN, $HCO3^-$, $PO4^{3-}$, CCI, MAP, MV, Hb, Cre, Alb, UO, APACHE II score, SOFA score, Cause of CRRT.

Discussion

In this study, we assessed the potential of WBC counts to predict mortality in a cohort of AKI patients in the ICU who received CRRT. Our study showed that WBC levels were associated with a high risk of all-cause mortality at 28 and 90 days in AKI patients who received CRRT. Compared with the reference level, the mortality risk was significantly higher in patients with low WBC levels (quartile 1). Furthermore, after adjusting for demographic variables, chronic disease, and clinical indicators, patients in the lowest WBC level group who received CRRT had 1.43-fold and 1.32-fold higher risk of all-cause mortality compared with the reference group at 28 and 90 days. GAM showed that a U-shaped relationship exists between the WBC counts and the mortality risk. These findings indicate that WBC counts have potential clinical application value for predicting the mortality rate of critically ill AKI patients receiving CRRT.

The association between WBC counts and mortality has been reported previously in observational studies [27–29], but the evidence is inconclusive. Consistent with the Women's Health Initiative study and the Taiwan area study [30,31], a U-shaped relationship between WBC counts and all-cause mortality was also observed in our study. Waheed et al. conducted a retrospective study involving 4,165 patients in ICU in the London area. They found that mortality rate was significantly higher (37.5%) in patients with low WBC counts compared with other groups [27]. Similarly, the results of ICU data from East Asia (N = 2,079) also supported that higher mortality rates are associated

 doi:10.20944/preprints202311.1224.v1

with lower levels of WBC counts [28]. Nevertheless, other studies, which included patients admitted to the ICU for infectious factors (N = 1,245) and non-infectious factors (N = 917), showed that elevated WBC counts correlate with an increased risk of mortality [29,32].

The discrepancies among studies may be due to variability in classification of WBC counts cut-off values, differences in cohort characteristics, and differences in adjustment covariates. For example, studies have employed different approaches to dividing the population when establishing the cut-off values for WBC counts. Whereas some studies used a range of normal WBC values (4.0-10.0 × 10^9/L) [27], others separated the population into quartiles [33]. The different grouping criteria used in these studies may have resulted in biased data and confounding variables between the groups, leading to variations in observations. In addition, there was a significant variation in age composition of participants in these studies. Age-related differences account for disparities in several aspects, including physical health, chronic comorbidities and immune competence [34,35]. Furthermore, failure to adjust for factors that may influence mortality could result in observations biased towards the null hypothesis [36]. However, in the present study, we utilized the quartile method for grouping to prevent imbalances between groups, and accounted for as many confounding variables as possible through univariate Cox regression.

Our study finds a U-shaped relationship between WBC count and all-cause mortality, patients with low WBC count being at the highest risk of death. Nonetheless, the underlying mechanism for this association remains unclear, and we refer to the possible explanations outlined in previous studies. The first hypothesis postulates that low WBC counts may indicate a weakened immune response, which makes patients more vulnerable to opportunistic pathogens. This vulnerability may then lead to an increased risk of infection and ultimately death [37]. The second hypothesis proposes that CRRT as one of the most applied blood purification therapies in the ICU, is frequently used to alleviate inflammatory cytokine storms [38]. However, these patients are accompanied by uncontrolled inflammatory responses and excessive intra-tissue infiltration of immune cells. Consequently, this leads to a decrease in WBC count levels in the peripheral blood [39]. Although these patients have lower WBC counts, they are simultaneously experiencing a more intense inflammatory response and are at a higher risk of death. The third theory is founded on the "immunosuppression" theory, which suggests that after exposure to multiple traumas, there is a substantial decrease in neutrophil numbers, chemotaxis and phagocytosis, leading to weakened immunity to external threats [40].

At present, the causal relationship between low WBC counts levels and all-cause mortality is unclear. Nevertheless, it is reasonable to conclude that early intervention for critically ill patients with low levels of WBC counts is essential, especially for those requiring CRRT upon ICU admission. Such interventions would optimize the utilization of medical resources, such as ICUs and healthcare staff, and to some extent, reduce the burden on healthcare professionals. At present, the relationship between low WBC levels and the true inflammatory profile in vivo in ICU patients undergoing CRRT is unknown. Therefore, further study is required to determine whether immunomodulatory strategies could reduce the risk of all-cause mortality in high-risk populations.

Moreover, our analysis revealed a higher risk of mortality in male patients receiving CRRT as compared to female patients, especially when evaluating all-cause mortality at 28 days as the primary endpoint. This observation agrees with previous studies that found gender differences in the risk of disease in many conditions [41,42]. Additionally, CCI showed a significant correlation with the risk of mortality in both short- and long-term studies, with patients scoring >2 in the CCI scale being at the highest risk of mortality. Patients with a high comorbidity burden are less likely to receive early diagnosis and treatment, increasing the risk of mortality in this group [43,44].

This study has several strengths: First, this was a national study with a large sample size, and nationally representative cohort of ICU patients from multiple centers in Korea. Second, it is the first study to investigate the relationship between WBC counts and all-cause mortality in critically ill AKI patients receiving CRRT. However, this study has some limitations. First, leukocyte subtypes or other important inflammatory markers such as cytokines and chemokines were not analyzed. Second, the study only examined overall mortality and did not analyze cause-specific mortality.

Conclusion

This multicenter study for the first time examined the relationship between WBC counts and all-cause mortality in ICU patients receiving CRRT. We found a U-shaped relationship between WBC counts and all-cause mortality, with the lowest WBC counts group having the highest risk of mortality. In addition, even after adjusting for potential confounding variables, the low WBC counts group still had the highest risk of mortality. Our study has important clinical implications for reducing the high mortality rate in ICU patients, especially in patients receiving CRRT. ICU providers should be aware of the increased risk of mortality in patients with very low WBC levels at the time of transfer and intervene early to improve the prognosis of this at-risk population.

Declarations

Ethics approval and consent to participate

Sun et al. shared the data for this study, which was approved by Yonsei University Health System, Severance Hospital, Institutional Review Board.

Authors' contributions: JX and HWH designed the study and conducted the data analysis. FH drafted the manuscript. JZ, ZQQ, YR, and SSC proposed critical revisions to the manuscript. All included authors made contributions to the manuscript and approved the version submitted. All authors read and approved the final manuscript.

Acknowledgements: We thank Sun et al. for their dedications and contributions.

Availability of data and material: This data is available in the Dryad database.

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