The Higher Level of Neutrophil – Lymphocyte Ratio (NLR) and Serum Syndecan-1 Based on Timeline (First, Sixth, and Twenty-Fourth Hour) in Sepsis-Induced Acute Kidney Injury

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Abstract

This study aims to analyze the difference between the NLR and serum syndecan-1 level at the first, sixth, and twenty-fourth hours with the incidence of acute kidney injury (AKI) and non-AKI in sepsis. We observed thirty-one adult sepsis patients who admitted to the emergency room and ICU of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from March 30, 2020, to July 5, 2020 (four-month), and differentiated into two groups, AKI group (n=14) and non-AKI group (n=17). All septic patients have been given the standard treatment based on Survival Sepsis Campaign 2018. The results showed that the NLR at 1st-hours, 6th-hours, and 24th-hours in the non-AKI group were lower than those in the AKI group (1st: 15.9±2.6 vs 33.0±11.3; 6th: 14.2±1.8 vs 19.3±3.8; and 24th: 11.6±1.3 vs 19.9±3.8) ($p < 0.00$). Almost NLR at every groups based on serial hours were decreasing, except in the AKI group, the NLR at the 24th-hour group was higher than the NLR at 6-hour group, but still lower than the NLR at 1st-hours. Serum syndecan-1 levels were lower in the non-AKI group than in the AKI group, at 1st, 6th, and 24th-hours, (1st: 532.5±72.0 ng/mL vs 597.2±85.8 ng/mL; 6th: 557.6±71.6 ng/mL vs 612.0±73.9 ng/mL; and 24th: 548.4±76.6 ng/mL vs 586.4±84.6 ng/mL) ($p < 0.05$). It can be concluded that the neutrophil-lymphocyte ratio (NLR) and serum syndecan-1 levels at 1st, 6th, and 24th hours is higher in the AKI compared to non-AKI groups in sepsis. Hence, NLR and serum syndecan-1 have a potential biomarkers for sepsis-induced AKI.

Keywords: *sepsis, acute kidney injury, neutrophils-lymphocytes ratio, syndecan-1, mortality*

Introduction

Sepsis is an emergency condition which described as a systemic immune response of the host that leads to organ damage and death caused by infection. Although the technology of hemodynamic monitoring and resuscitation improved significantly, it still does not change a lot the morbidities and mortalities evidence [1,2]. The worldwide incidence of sepsis is around 707 cases per 100,000 people with an in-hospital mortality rate of 20 – 43% [3]. The incidence of Sepsis-Induced acute kidney injury (S-AKI) is around 19 million cases worldwide and about 1 in 3 cases of sepsis will become acute kidney injury (AKI) with a significantly higher mortality rate of around 44% [4].

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Neutrophils and lymphocytes have an important role in inflammation and tissue damage. The role of neutrophils and lymphocytes in AKI has been recognized as an important modulator of the immune response. Ischemic, nephrotoxic, and AKI caused by endotoxemia are associated with an increase in neutrophils influx into the kidney [5]. Increase in the neutrophil-lymphocyte ratio (NLR) has been reported to be a useful prognostic marker for several diseases, include for sepsis. The NLR is an examination that is simple, easy to do and inexpensive, so the use of the results of the NLR examination as a predictor of the incidence of AKI is promising and has the potential as a parameter to differentiated the degree of AKI in sepsis [6].

Sepsis could cause endothelial injury, of which Syndecan-1 is one of the biomarkers, leading to endothelial glycocalyx degradation, vasodilation, capillary leakage, and hypoperfusion leading to AKI [7–9]. Increased serum syndecan-1 levels in sepsis and septic shock patient is associated with higher Sequential Organ Failure Assessment (SOFA) score and Acute Physiology And Chronic Health Evaluation *II* (APACHE II) score, increased length of stay, severity, and mortality [10–12]. Nevertheless, the NLR and serum Syndecan-1 level based on timeline in S-AKI to predict the recovery or severity are unclear. Hence, the objective of this research is to analyze the difference the NLR and serum Syndecan-1 level at the first, sixth, and twenty-fourth hours with the incidence of AKI and non-AKI in sepsis, which is cheaper and more practical.

Material and Methods

Approval for this research has been obtained from Health Research Ethics Committee Dr. Soetomo General Academic Hospital (No.1807/KEPK/I/2020).

Samples

Thirty-two adult sepsis patients who admitted to the emergency room and ICU of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from March 30, 2020, to July 5, 2020 (four-month), and had met the inclusion criteria and not met exclusion criteria.

The inclusion criteria were 18th – 65th year sepsis patients who meet the criteria based on The Third International Consensus Definitions for Sepsis and Septic Shock 2016 (Sepsis-3). A critically ill septic patient not eligible to make their own decisions, so the guardian and/or patient's family (at least 2 persons) stated their willingness that the patient was included in the study by signing information for consent and an informed consent approval.

The exclusion criteria were the evidence of the history of vascular disorders, chronic kidney disease, dyslipidemia or using dyslipidemia drug in the statin or non-statin class. A diagnosis of sepsis for referred hospital previously and/or one-hour time zero of sepsis diagnose had passed after the triage.

All septic patients gave the standard treatment based on sepsis standard management protocol at Dr. Soetomo General Academic Hospital. We observed and collected the data in the first twenty-four hours, such as the history of the patient, comorbid, SOFA Score, APACHE Score, physical examination, vital sign, fluid resuscitation, fluid balance, routine laboratory – radiology examination, and diagnosis. Blood sampling was taken for NLR and syndecan-1 serum at first, sixth- and twenty-fourth hours of treatment. NLR was measured by dividing the absolute neutrophil with lymphocyte count from complete blood count result. For syndecan-1 sampling, the blood was centrifuged then examined with *Cusabio Human Syndecan-1/CD138(SDC1) ELISA Kit* reagent. Thirty-one samples were included in this research. AKI was diagnosed by Kidney Disease: Improving Global Outcomes (KDIGO) criteria, using creatinine and urine productions parameter, at early sepsis were diagnosed, and evaluated in 24 h of treatment.

Statistical Analysis

The data were analyzed with SPPS software. Normality test was performed to evaluate data

distribution. The Independent-T test and the multivariate – ANOVA were used to analyze the difference between groups and serial variables. The Chi-square and the wilcoxon-mann whittney test were applied when it was not normally distributed or the data was in ordinal scale.

Results

In the AKI group, men are dominant compare to women (10 vs 4) and in the non-AKI group women more dominant than men (5 vs 12) (p = 0.022). Age, weight, height, and BMI were the same in both groups and there was no statistically significant difference. The SOFA score in the AKI group was higher than the non-AKI group, both at admission evaluation and at 24-hour evaluation (admission p = 0.003; 24 hours p = 0.001). There was no significant difference between the two groups for the lactate level. Mortality in the AKI group was 38.7% higher than the non-AKI group with 22.6%. In the AKI group, the highest mortality was at 7 days mortality (19.4%) and in the non-AKI group, 7-day mortality was much lower (9.7%). Mortality and survival in the two groups were statistically significant (p = 0.023) (Table 1).

Table 1. Sample Characteristic.

Variable	Group		p value
	Non-AKI (n=17)	AKI (n=14)	
Sex			0.022*
Man (n)	5	10	
Women (n)	12	4	
Age (year)	46.7±14.7	48.9±13.8	0.218
Body Weight (Kg)	60.0±8.6	62.5±10.5	0.094
Body Hight (cm)	158.3±8.1	160.9±7.6	0.098
BMI	24.1±4.0	24.1±3.7	0.293
SOFA score (admission)	4.7±2.0	7.1±2.1	0.003*
SOFA score (24 hour)	4.7±1.9	7.9±3.3	0.001*
APACHE II score	17.1±7.3	23.3±4.6	0.003*
Lactate 1st hour (mmol/L)	2.0±1.5	2.7±1.6	0.292
Lactate 6th hour (mmol/L)	2.0±1.8	2.1±1.3	0.474
Overall Mortalities (19) +	7(22.6%)	12 (38.7%)	0.023+
7-day mortality (n)	3 (9.7%)	6 (19.4%)	
28-day mortality (n)	4 (12.9%)	5 (16.1%)	
>28-day mortality (n)	-	1 (3.2%)	
Survive / Discharge (12)	10 (32.3%)	2 (6.5%)	

Note: * Chi-square (significant; p<0.05), + Mann-Whitney Test (significant; p<0.05)

Table 2 shows that the NLR at 1st and 6th hours in the AKI group were higher than in the non-AKI group, but the NLR at 24 hours in the non-AKI group was higher than in the AKI group (p<0.05) (Table 2). The NLR in AKI decreased at its lowest point in the 6th hour and increased again in the 24th hour, although only slightly. However, in the non-AKI case, there was a downward trend from the 6th hour and continued to fall at the 24th hour.

Table 2. Neutrophil-lymphocyte ratio.

Variable	Group a		p value
	Non-AKI (n=17)	AKI (n=14)	
NLR 1st hours	15.9±2.6	33.0±11.3	0.000*
NLR 6th hours	14.2±1.8	19.3±3.8	0.000*
NLR 24th hours	11.6±1.3	19.9±3.8	0.000*

Note: [a] Mean (Standard Errors), * Multivariate ANOVA test (significant; p<0.05).

Serum syndecan-1 levels were higher in the AKI group than in the non-AKI group, both at 1st, 6th, and 24th hours, and this difference was significant (p <0.05) (Table 3). The highest levels occurred at 6th hours or after initial resuscitation, in both the AKI and non-AKI groups. In the non-AKI group, the impressive serum syndecan-1 levels continued to increase and this was not found in the AKI group.

Table 3. Serum syndecan-1 level.

Variable	Group a		p value
	Non-AKI (n=17)	AKI (n=14)	
Syndecan-1 1st hours (ng/mL)	532.5±72.0	597.2±85.8	0.000*
Syndecan-1 6th hours (ng/mL)	557.6±71.6	612.0±73.9	0.000*
Syndecan-1 24th hours (ng/mL)	548.4±76.6	586.4±84.6	0.000*

Note: [a] Mean (Standard Errors), * Multivariate ANOVA test (significant; p<0.05).

Discussion

Sepsis Has A Complex And unique pathophysiology, so AKI in sepsis is a syndrome that is different from another AKI. Sepsis is defined as a syndrome of organ dysfunction due to infection so that infection with AKI is the same as sepsis [4]. AKI in sepsis is associated with poor clinical conditions. Compared with other critically ill patients with AKI, septic patients with AKI had a higher risk of death in hospital (odds ratio: 1.48) and a longer hospital stay compared with AKI from other causes (37 vs 21 days) [13]. In this study, the mortality rate in sepsis with the AKI group was high (38.7%) with a survival rate of only 6.5% compared to the non-AKI group (22.6%) with a survival rate of 32.3%. This difference from the statistical test results was significantly different (p = 0.023) indicating that sepsis with AKI had a higher mortality risk than sepsis without AKI. Sepsis with AKI had the highest mortality rate before day 7 (19.4%) followed by mortality before day 28 (16.1%).

Increased NLR can occur in septic patients with AKI [6]. The increase in NLR during the first 48 hours was associated with the incidence of organ failure in critically ill male trauma patients (OR 2.06 (1.04-

4.06), p = 0.04) [14]. However, in our study, the highest NLR was found at the first hour of sepsis (p = 0.000). This phenomenon can be interpreted that when a patient is diagnosed with sepsis, there is a severe systemic inflammatory process and during the NLR evaluation at the 6th and 24th hours, where the patient has received standard sepsis management therapy (based on Survival Sepsis Campaign Guideline 2018), such as giving fluids, maintaining MAP, giving antibiotics and so on, causing a decrease in the systemic inflammatory process, even though the systemic inflammatory process is still in a dangerous phase. In another retrospective study of 13,678 AKI patients who were critically ill, it showed that an NLR higher than 12.14 was a predictor of all causes of death (HR 1.83 (1.66-2.02), p <0.0001) [14]. Although the standard limit values for NLR have not been determined [15].

Sepsis can cause endothelial injury and cause endothelial cell dysfunction which can lead to vasorelaxation or vasodilation, impaired vascular permeability and extravasation of leukocytes or neutrophils [16]. Syndecan-1 is one of the components forming the endothelial glycocalyx and is one of the main proteins that attach to the apical membrane of endothelial cells [9,17]. Serum syndecan-1 levels can be measured at admission which is independently associated with severe AKI. The accuracy level of serum syndecan-1 in non-septic conditions for the diagnosis of severe AKI is moderate (area under the ROC curve, 0.77; 95% confidence interval, 0.68-0.85) [18]. Syndecan-1 serum levels have been associated with an increased risk of death by 90 days of mortality. In addition, glycocalyx and endothelial injury on admission to the ICU was associated with an increased risk of AKI and death. Higher serum syndecan-1 levels have been associated with AKI in a study among 175 septic patients. Although serum syndecan-1 levels in the study were higher among AKI patients within 12 hours and syndecan-1 serum levels were not independently associated with AKI [18–20]. Our research showed that there was a significant difference (Multivariate ANOVA test; p = 0.000) between septic patients with AKI or without AKI, both at the 1st hour, 6th, hour and 24th hour. However, the highest Serum syndecan-1 level was obtained at 6 hours, after fluid resuscitation was given. This is in contrast to the findings of Inkinen et al. who found no significant difference in syndecan-1 serum levels associated with fluid balance during the first 24 hours [20].

Conclusion

The conclusion of this study is the neutrophil-lymphocyte ratio (NLR) is higher in the 1st and 6th hours in AKI, however it is lesser at 24th hours in the AKI in sepsis. The serum syndecan-1 levels at 1st, 6th, and 24th hours were greater in the AKI in sepsis. Therefore, the NLR and serum Syndecan-1 level based on timeline have a potential to become biomarkers for sepsis-induced AKI. However, further research is needed, especially for direct correlation with other markers or clinical symptoms.

Author Contributions

Lila Tri Harjana assisted in conducting the research, performed the statistical analysis, and data visualization and wrote the manuscript. Eddy Rahardjo designed and conducted all of the research. Windhu Purnomo performed the statistical analysis and data visualization. Nancy Margarita Rehatta designed all of the research. Lilik Herawati performed the statistical analysis and data visualization, and wrote the manuscript. All authors have read and approved of the final manuscript.

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