DIFFERENCE BETWEEN NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) BETWEEN LUPUS PATIENTS WITH AND WITHOUT NEPHRITIS

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Abstract

Neutrophil to lymphocyte ratio (NLR) can be a potential biomarker for disease activity and prognosis in the development of systemic lupus erythematosus (SLE). This study aims to determine if there is a difference between NLR values between lupus patients with nephritis and lupus patients without nephritis. The subjects of this study were patients with SLE diagnosis in Dr. Hasan Sadikin General Hospital Bandung from January to December 2019. The selected statistical test of difference was a non-parametric Mann-Whitney test, the analysis of the receiver operating curve (ROC) was conducted to obtain the cut-off value of NLR in predicting lupus nephritis. Data processing was conducted using SPSS® software version 20.0. The total obtained data for lupus patients with and without nephritis was 156 patients. There was a significant NLR difference between lupus patients with nephritis and without nephritis (the median NLR for a lupus patient with nephritis was 4.20, while the median NLR for a lupus patient without nephritis was 2.82, with a p-value of <0001, the cut-off value of NLR was >3.52 with the sensitivity of 67.6%, specificity of 67.1% in distinguishing between lupus with nephritis and without nephritis. in this study, it was obtained that the difference between NLR in lupus patients with and without nephritis yielded a p-value of <0.05. However, NLR has not been determined as a biomarker parameter to determine disease activity (flare-ups) and prognosis.

Keywords: Lupus nephritis, neutrophil to lymphocyte ratio (NLR)

Main Text

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease associated with autoimmune reactions. The main characteristic of this disease is the production of a cluster of heterogenous autoantibodies against autoantigens in cells or the blood circulation system. The immune system in SLE patients is abnormally activated by autoantigens, which will deposit the immune complex and activates the complement system, followed by chronic inflammation. This antigen-antibody complex may occur in organs such as the kidney, heart, joint, and liver.[1]

About 50–80% of SLE patients will develop lupus nephritis, which remains the most severe SLE manifestation.[1,4] Lupus nephritis is defined as clinical and laboratory manifestation fulfilling American College of Rheumatology criteria (occurred after the diagnosis of SLE has been established, with persistent proteinuria of >0.5 gram/day or more than 3+ using dipstick test, and/or cellular cast, including red blood cells, hemoglobin, granular cast, tubular cast or mixed casts). Lupus nephritis is a severe SLE manifestation and is associated with a risk of terminal-stage kidney failure to death. Thus, early diagnosis and accurate monitoring is a challenge in managing SLE patients.[1,2]

Neutrophils are a cell with an important role in the pathogenesis of SLE. Neutrophils are a leukocyte with the highest amount in the body with the most important role in almost all major aspects of the human immune system. Neutrophil plays a role in phagocytosis, oxidative reaction, and formation of neutrophil extracellular traps (NET), all these aspects are vital in the body's immune defense system, and disruptions in these functions are also associated with the development of autoimmune disease. Altered tolerance towards autoantigens and increased neutrophil apoptosis are the main causative factors for SLE. Release of NET by neutrophils and activation of plasmacytoid dendritic cells directs chronic production of interferon-α in SLE. After phagocytosis by antigen-presenting cells (APC), such as dendritic cells (DC), this autoantigen is processed and presented on the surface of the cell. T-cell recognizes this autoantigen and stimulates B-cell to secrete autoantibody. This autoantibody may react with the appropriate antigen in the blood circulation system or may cross-react with an intrinsic glomerular antigen which will be deposited in the glomerular basement membrane (GBM) of the kidney. The blood circulation system and intrinsic immune complex (IC) will activate complements and causes infiltration of inflammatory cells, activation of coagulation factors and inflammatory mediators, and eventually, causes kidney damage.[3,23]

Neutrophils and lymphocytes play important roles in lupus nephritis, and their values change with the worsening and remission of systemic inflammatory response. Neutrophil and lymphocyte counts are routinely administered on daily practice and their examination is very easy to conduct, making the NLR easily calculated on routine blood examination. Increased NLR is associated with increased cytokines and inflammatory processes in SLE. An imbalance in the number of neutrophils and lymphocytes contributes to the development of SLE. NLR is increased in SLE patients and may indicate inflammatory response and disease activity.[4]

Neutrophil to lymphocyte ratio (NLR), which is calculated by dividing the number of absolute neutrophil counts by the number of absolute lymphocyte counts, is a potential biomarker for disease activity and prognosis in the development of SLE. Neutrophil to lymphocyte count is an inflammatory index obtained from routine hematology examination, requires minimum cost, is readily available, and is easily examined. During inflammation, the number of neutrophils and lymphocytes will be temporarily altered. The absolute neutrophil count will increase, while the absolute lymphocyte count will decrease in autoimmune diseases. Neutrophil to lymphocyte ratio may be utilized as a biomarker in several immunological diseases, including inflammatory bowel disorder, psoriasis, and Sjögren syndrome.[5,6]

Based on this theory, this study aims to determine if there is a difference between NLR values between lupus patients with nephritis and lupus patients without nephritis

Methods

This was a cross-sectional study with an observational analytic design. Data were obtained retrospectively using secondary data obtained from Laboratory Information System (LIS) and patient medical records.

The subjects of this study were patients with SLE diagnosis in Dr. Hasan Sadikin General Hospital Bandung from January to December 2019. The definition of lupus nephritis in this study was all patients with established SLE diagnosis based on ACR criteria with proteinuria of more than +3 using the dipstick method.

The inclusion criteria for study subjects were SLE patients who were evaluated for routine blood count and urine protein examination at the same time during outpatient follow-up visits. The exclusion criteria for this study were patients with incomplete medical record data.

The normality test for numerical data was conducted using the Kolmogorov-Smirnov test. Based on the normality test, it was known that age, creatinine, and NLR data were not normally distributed, hence, the selected statistical test of difference was a non-parametric Mann-Whitney test, while the test of difference for gender as categorical data was examined using Chi-square test. The analysis of ROC was conducted to obtain the cut-off value of NLR in predicting lupus nephritis. Data processing was conducted using SPSS® software version 20.0.

Results and Discussion

The total obtained data for lupus patients with and without nephritis was 156 patients. The characteristics data of lupus patients with and without nephritis were presented in Table 1 below:

Table 1 Characteristics and Difference between Lupus Patients with and without Nephritis

	Lupus Nephritis n=74	Lupus without Nephritis n=82
Sex, n (%)		
Male	5 (6.8)	5 (8.5)
Female	69 (93.2)	75 (91.5)
Age (years)		
Median (min-max)	17 (11 – 42)	14 (3 – 45)
Creatinine (mg/dL)		
Median (min-max)	1.06 (0.37 – 10.19)	0.55 (0.03 – 1.31)

Table 1 showed that lupus patients with and without nephritis were more frequent in females (93.2% on lupus with nephritis, 91.5% on lupus without nephritis) compared to males (6.8% on lupus with nephritis, 8.5% on lupus without nephritis). There was a median age of 17 years (11-42 years), a median creatinine level of 1.06 mg/dL (0.37-10.19 mg/dL) on lupus nephritis patients; meanwhile, the median age was 14 years (3-45 years), median creatinine level of 0.55 mg/dL (0.03-1.31 mg/dL) on lupus patients without nephritis.

The difference in NLR between lupus patients with nephritis and without nephritis was presented in Table 2 below:

Table 2 NLR difference between Lupus Patients with and without Nephritis

	Lupus Nephritis n=74	Lupus without Nephritis n=82	p-value
NLR			
Median (min-max)	4.20 (0.19 – 47.50)	2.82 (0.67 – 14.00)	**<0.001**

Note: Analysis using the Mann-Whitney test

Table 2 showed a significant NLR difference between lupus patients with nephritis and without nephritis (median NLR for lupus patients with nephritis was 4.20, while median NLR for lupus patients without nephritis was 2.82, with a p-value of <0001).

Based on the receiver operating curve (ROC) with an area under the curve (AUC) of 0.705, the cut-off value of NLR was >3.52 with a sensitivity of 67.6% and specificity of 67.1% in distinguishing between lupus with nephritis and without nephritis. The figure below presented the ROC NLR in predicting lupus nephritis:

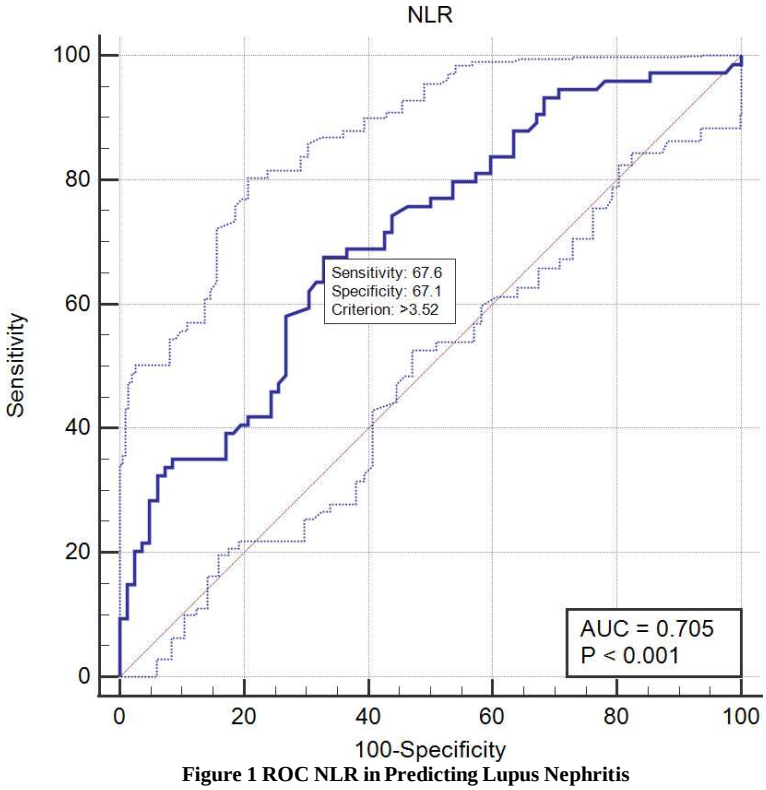

Figure 1 ROC NLR in Predicting Lupus Nephritis

The characteristics of subjects in this study were mostly female (93.2% on lupus with nephritis and 91.5% on lupus without nephritis). This result was in accordance with a study result by Wallace, et al. in 2007 that obtained a female-to-male ratio of 9:1. A study by Rahman, et al. in 2008 stated that more than 80% of patients with SLE were affected by reproductive age, ranging from 15 – 40 years; a similar result was also observed on this study in which the patients age ranged from 11 – 42 years on lupus with nephritis patients and 3 – 45 years on lupus without nephritis.[9]

The most severe and frequent manifestation of SLE was lupus nephritis. in this study, it was known that the difference between creatinine value between lupus patients with nephritis and without nephritis yielded a p-value of <0.001. A similar result was also presented by Abdulrahman, et al. in 2019 which stated that creatinine in lupus nephritis was higher (1.5 ± 0.3) compared with lupus patients without nephritis (0.72 ± 0.1). Creatinine is an indicator of kidney function and in lupus nephritis, kidney function was notably declining.[1]

in this study, the NLR value was higher in lupus nephritis patients compared to lupus patients without nephritis (p <0.001). This result was in accordance with a study result by Li, et al. in 2015 which stated that NLR value was higher in lupus patients with complication of lupus nephritis compared to patients without complication (p <0.001),[16] thus, NLR was a potential marker that may represent kidney

involvement in patients with SLE.

This study yielded an AUC of 0.705 with an NLR cut-off value of >3.52 which may predict lupus nephritis with sensitivity of 67.6% and specificity of 67.1%. This result was in contrast with the result obtained by Soliman, et al. in 2018 which presented an AUC of 0.747 with NLR cut-off value of 2.2 to predict lupus nephritis with sensitivity of 90% and specificity of 50%.[12] This difference in result was presumably because the lupus nephritis group in the study by Soliman, et al. already had their diagnosis confirmed by kidney biopsy, thus increasing the result accuracy compared with this study. Moreover, the population in this study was not categorized based on the presence of flare-ups (Disease Activity Score).

NLR has several advantages as an index of inflammation. First, NLR was superior to absolute neutrophil and lymphocyte count because the latter was affected by many factors, including dehydration, overhydration, and improper blood specimen handling. Second, NLR may represent an increase of neutrophils and a decrease of lymphocytes in the body's immune system.[6]

On SLE patients, higher NLR values showed the presence of more severe pathological damage.[13] Pathologic changes in the kidney should be diagnosed with kidney biopsy; however, this procedure opposed several risks, among them was bleeding which may cause a perirenal hematoma. In contrast, NLR could be obtained from routine blood count examination, which was relatively cheaper, quicker, and more readily available.[5]

Lupus nephritis frequently occurred in SLE patients and causes kidney damage in 50-80% of patients, with the presence of proteinuria and cells on renal tubules observed at the beginning and along the course of the disease.[13,14] About 10 – 30% of lupus nephritis developed into end-stage renal failure.[21] This risk remains unchanged for the last three decades. Lupus nephritis patients have poor long-term quality of life and prognosis. Moreover, about 27-66% of patients in remission may experience flare-ups in the future. Thus, a safer, more convenient, and quicker indicator was promptly required to determine the level of kidney inflammation in hopes to prevent recurrence and protect kidney function.[14,16]

Lupus nephritis was marked by the deposition of the immune complex in the kidney, which would activate the complement system and inflammatory cells such as neutrophils, monocytes, eosinophils, and T-lymphocytes. This immune complex may stimulate cell phagocytosis and release reactive oxygen species (ROS) which may damage body tissue. This basic theory is the mechanism behind the association between inflammatory markers and disease activity in SLE.[1]

Inflammasome NLRP3 plays an important role in lupus nephritis. Immune complex, interference factor I, and NET in tissue may increase the activity of NLRP3. In SLE patients, excessive macrophage reaction may escalate the activity of inflammasome to increase inflammatory cytokines. Inflammasomes may also reduce autoantibodies in the patient, causing rapid deterioration of nephritis. Recent studies showed that the causative mechanism of lupus nephritis was due to impaired immune systems on target organs and non-immune factors which eventually would damage the organ through the combination of target organ resistance and local inflammatory response.[8,22]

Leukocytes play an important role in the inflammation process and neutrophils made the most of leukocytes.[12,13] Neutrophils as part of the innate immune system, also secreted several different cytokines and inflammatory mediators as a response towards antigens or tissue injury.[16] Neutrophils would develop functional impairment, including reduced phagocytic and lysosome activity, production of reactive

oxygen species, increase in adhesion molecules, and cellular aggregation and intracellular activation which would worsen SLE.[14,21]

There were several systemic oxidative stress responses in the body. In the kidney, T-lymphocyte would infiltrate the interstitial tubules area of the kidney and then, release angiotensin. This condition caused chronic inflammation, damage, and necrosis of kidney tissue, which was directly associated with an increase of neutrophils in internal circulation. A decrease in lymphocyte count may cause an increase in neutrophil count and a prolonged, chronic inflammatory response.[8]

The limitation of this study was the absence of Disease Activity Score on medical records, hence it was unknown whether lupus nephritis patients with high NLR value were on flare-up (in terms of disease activity) and it was also unknown how long the patient has been diagnosed with lupus nephritis and their history of treatments.

Conclusion and Suggestion

In this study, it was obtained that the difference between NLR in lupus patients with and without nephritis yielded a p-value of <0.05. However, NLR has not been determined as a biomarker parameter to determine disease activity (flare-ups) and prognosis.

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