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Original Article

Neutrophil To Lymphocyte Ratio as A Marker of COVID-19 Disease Severity in Banda Aceh

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Abstract

Background: *Patients with severe COVID-19 always coincide with high mortality rate, meanwhile, inflammatory immunity parameters in COVID-19 infection such as Neutrophil to Lymphocyte Ratio (NLR) reflect the infection progress. These are used by clinicians for early identification of patients at high risk or to determine when it is a serious disease. This study aims to determine the effectiveness of NLR as a marker of COVID-19 pneumonia severity.*

Method: *This study was conducted using a retrospective cross-sectional analytical design at the Regional General Hospital of DR. Zainoel Abidin Banda Aceh from June to September 2020. Patients' demographic characteristics, comorbidities, clinical manifestations of COVID-19 infection, chest x rays, examination of blood samples at admissions such as leukocytes, lymphocytes, neutrophils and NLR were extracted from medical record data. The patients were divided into four groups according to the disease severity, namely mild, moderate, severe and critical.*

Result: *A total of 105 medical records were collected for COVID19 patients, meanwhile, 51 to 70 years was the largest age group (60.8%) with twice male than female. Moreover, fever, cough, shortness of breath and weakness are the most common symptoms found in treated patients while almost two-thirds of the patients have bilateral pneumonia. Generally, the levels of leukocytes, neutrophils, NLR were found to increase while the levels of lymphocytes decreased, in addition, more than half of the COVID19 patients were severe. There was a strong relationship between an increase in NLR levels and COVID-19 disease severity (α: 0.05; P=0.001)*

Conclusion: *Based on the results, NLR is applicable as an early inflammatory marker which reflects severe and critical COVID19 infection and also suitable as an objective basis for early identification and management of severe COVID-19 pneumonia.* ***(J Respirol Indones 2021; 41(4): 272–8)***

Keywords: *Neutrophyl Limfosit Ratio, Severity, COVID19*

Neutrophil To Lymphocyte Ratio sebagai Marker Derajat Keparahan COVID-19 di Banda Aceh

Abstrak

Latar belakang: *Penderita COVID-19 berat selalu memiliki tingkat mortalitas yang tinggi, sementara itu parameter imunitas inflamatorik pada infeksi COVID-19 seperti Rasio Neutrofil Limfosit (RNL) mencerminkan progresivitas infeksi. Hal ini digunakan oleh klinisi sebagai identifikasi awal pada pasien dengan risiko tinggi atau untuk menentukan tingkat keseriusan penyakit. Penelitian ini bertujuan untuk mengetahui efektivitas RNL sebagai penanda tingkat keparahan pneumonia COVID-19.*

Metode: *Penelitian ini menggunakan desain analitik potong-lintang retrospektif di Rumah Sakit Umum Daerah Dr. Zainoel Abidin Banda Aceh pada bulan Juni hingga September 2020. Karakteristik demografi pasien, komorbiditas, manifestasi klinis infeksi COVID-19, rontgen dada, pemeriksaan sampel darah pada saat rawat inap seperti leukosit, limfosit, neutrofil dan RNL didapatkan dari data rekam medis. Pasien dibagi menjadi empat kelompok sesuai dengan tingkat keparahan penyakitnya, yaitu ringan, sedang, berat dan kritis.*

Hasil: *Sebanyak 105 rekam medis pasien COVID19 dikumpulkan, dimana kelompok umur terbesar adalah 51-70 tahun (60,8%) dengan jumlah laki-laki dua kali lebih banyak dibandingkan perempuan. Selain itu, demam, batuk, sesak napas dan kelemahan adalah gejala yang paling umum ditemukan pada pasien yang dirawat, sementara hampir dua pertiga pasien menderita pneumonia bilateral. Secara umum kadar leukosit, neutrofil, RNL ditemukan meningkat sedangkan kadar limfosit menurun, selain itu, lebih dari separuh penderita COVID19 tergolong parah. Terdapat hubungan yang kuat antara peningkatan kadar RNL dan keparahan penyakit COVID-19 (α: 0,05; P=0,001).*

Kesimpulan: *Berdasarkan hasil penelitian, RNL dapat digunakan sebagai penanda awal inflamasi yang mencerminkan infeksi COVID19 yang parah dan kritis serta cocok digunakan sebagai dasar yang objektif untuk identifikasi awal dan manajemen pneumonia COVID-19 berat.* ***(J Respirol Indones 2021; 41(4): 272–8)***

Keywords: *Neutrophyl Limfosit Ratio, Severity, COVID19*

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INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with increasing morbidity and mortality in Indonesia.[1] The Reverse Transcription Polymerase Chain Reaction (RT-PCR) examination is the standard for diagnosing this disease. However, the tests are very limited, expensive, rare, and difficult to perform as the tests are generally carried out in the provincial capital laboratories and there is no established World Health Organization (WHO) algorithm to clearly determine COVID-19 cases.[2] Therefore, most clinicians make an initial diagnosis based on clinical, laboratory and radiological data.[3] When the initial outbreak occurred in China, it was difficult to identify patients suspected of being infected with COVID-19, therefore, initial diagnosis and management were based on simple clinical data.[4]

Several studies have indicated the role of inflammation in the development of viral pneumonia such as COVID-19. White blood cells or leukocytes differential count, especially neutrophils and lymphocytes, neutrophils-to-lymphocytes ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are systemic inflammation markers.[5] Neutrophils are the first leukocytes which migrate from the blood to injured or infected sites to kill pathogens through cytotoxic mechanisms.[6]

Besides, in inflammatory conditions, neutrophils extracellular traps (NETs) is formed actively from neutrophils to trap and kill pathogens.[6] Therefore, the complete blood count (CBC), especially neutrophils and lymphocytes are inexpensive clinical indicator markers of inflammation with fast circulation time and are simultaneously specific and sensitive.[7] Neutrophils increased with bacterial infection and lymphocytes are reduced during viremia. The serial assessment of the two parameters are of great help in assessing COVID-19 infection.[8]

The NLR is a simple blood test that is easily applied in daily clinical practice. It is cost effective and useful as an assessment and consideration for treating patients.[9] Meanwhile, the NLR value is very important for the treatment of inflammation and also used as a predictive value of mortality in COVID-19 patients.[10] It also provides an objective side for identification in severe COVID-19 patients. Furthermore, increased NLR is used as an early warning sign of severe COVID-19 symptoms and as an independent prognostic marker.[11] NLR is a biomarker of widespread inflammatory conditions, which is used to reflect disease severity.[12]

Most of the NLR studies have been reported in China, Europe, and in South Asian, while very few studies in Indonesia.[7,11] Therefore, this study aims to determine the effectiveness of NLR on COVID-19 severity.

METHOD

This study used a retrospective cross-sectional method by taking medical records of confirmed COVID-19 patients that received treatment at the Respiratory Intensive Care Unit (RICU) as well as New-emerging and Re-emerging Infectious Diseases (PINERE) at the Regional General Hospital, DR. Zainoel Abidin, Banda Aceh, from June to September 2020. This study was reviewed and approved by the Institutional Review Board of the School of Medicine, Syiah Kuala University, Banda Aceh (297/EA/FK-RSUDZA/2020) together with the National Health Research and Development Ethics Commission (KEPPKN) of the Indonesian ministry of health (#1171012P).

Demographics data (gender, age and occupation), comorbid (hypertension, diabetes mellitus (DM), coronary heart disease), lung diseases (asthma or chronic obstructive pulmonary disease (COPD)), and clinical symptoms were collected and recorded on the data collection form. In addition, during hospital admission, an evaluation of the data from chest X-ray examination and laboratory tests of blood samples was also carried out according to standard operating procedures. In this study, the NLR was used as a marker of inflammation which results from the distribution of total neutrophils to lymphocytes.[13]

Disease severity is divided into four categories, namely mild, moderate, severe, and critical based on WHO guidelines.[14]

$$NLR = \frac{Absolute\ Number\ of\ Neutrophil}{Absolute\ Number\ of Lymphocyte}$$

Exploratory statistical analysis was performed to assess potential patient characteristic variables including demographics, clinical symptoms, laboratory results and chest X-rays. For statistical analysis, the NLR values were divided into two categories, namely low and high. The value is low when NLR level is less than 13.51 and high when it is greater than 13.51. To test the effect of NLR levels and COVID-19 severity, the Spearman test was used. The significance for all data analyzed was α = 0.05. All statistical analysis were carried out using the Statistical Package for Social Sciences (SPSS) for Windows version 25.0 (IBM SPSS Inc., USA).

RESULT

A total of 105 medical records for COVID-19 patients were collected. The 51 to 70 years age group was the most predominant age group found (60.8%). Based on gender, the male treated patients were twice as much as the females. Most of the occupations are government and private employees that are usually active and have indoor activities, which allows a greater possibility for work-related transmission. Fever, cough, shortness of breath and weakness are the most common symptoms found in treated patients. Almost two-thirds of the patients have bilateral pneumonia, meanwhile, patients treated without pneumonia symptoms or had normal x-ray results were only 6 to 7 out of 100. The most common comorbids are DM and hypertension. The complete demographic characteristics of the study are shown in Table 1.

The treated patients had a systolic pressure of 131 to 132 mmHg and a diastolic pressure of 78 to 79 mmHg. Generally, patients suffered from tachypnoea with a frequency range of breaths between 18 and 36 times per minute. Although the patient's body temperature was still within normal, the average was almost 37°C. The average oxygen saturation was 88 to 89% indicating that the patient had mild hypoxemia. The levels of leukocytes, neutrophils and NLR were found to increase while the levels of lymphocytes decreased (Table 2).

Table 1. Demographic characteristics of the study

Characteristics	Number	Percentage
Age		
21-40 years	6	5.7
41-60 years	62	59
61- >70 years	37	35.3
Gender		
Male	72	68.6
Female	33	31.4
Occupation		
Civil servants	51	62.8
Private employees	35	33.4
Unemployed	16	15.2
Clinical manifestations		
Fever	88	83.8
Cough	87	82.9
Shortness of breath	77	73.3
Weakness	59	56.2
Nausea	19	18.1
Sore throat	18	17.1
Headache	17	16.2
Anosmia	11	10.5
Chest X-ray		
Normal	7	6.7
Pneumonia	29	27.6
Bilateral pneumonia	69	65.7
Comorbid		
DM	51	48.6
Hypertension	30	28.6
Obesity	18	17.1
COPD	7	6,7
Coronary Artery Disease	6	5.7
Chronic Kidney Disease	4	3.8
Chronic Heart Failure	1	1.0
The severity of COVID-19		
Mild	7	6.7
Moderate	18	17.1
Severe	42	40.0
Critical	38	36.2

Table. 2 Vital signs and laboratory of COVID-19 patients

Parameter	Variable	n	Min.	Max.	Mean	SD
Vital signs	Systolic	105	83.00	188.00	131.68	19.97
	Diastolic	105	48.00	110.00	78.38	10.88
	Heart rate	105	61.00	138.00	95.09	13.16
	Respiratory rate	105	18.00	36.00	26.61	5.01
	Temperature	105	35.70	38.80	36.91	0.52
	O_2 saturation (without O_2 supplementation)	105	60.00	99.00	88.54	7.54
Laboratory	Hemoglobin	105	4.20	18.00	13.09	2.39
	Leukocytes	105	1.100	34.400	11.465	6.071.28
	Segment neutrophils	105	43.00	97.00	82.75	10.84
	Lymphocytes	105	2.00	41.00	11.07	8.94
	NLR	105	1.05	48.50	13.51	10.90

Table 3. Relationship between NLR levels based on the severity of COVID-19

NLR levels	Severity								Total		*P*	r
	Mild		Moderate		Severe		Critical					
	n	%	n	%	n	%	n	%	n	%		
Low	7	11.3	18	29.0	27	43.5	10	16.1	62	59	0.001	0.69
High	0	0	0	0	15	34.9	28	65.1	43	42		
Total	7	6.7	18	17.1	42	40	38	36.2	105	100		

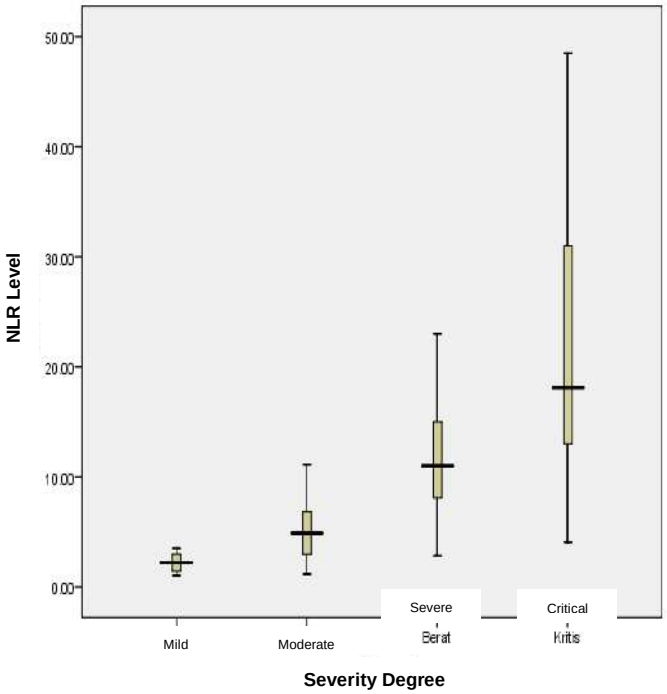

Figure 1. Boxplot diagram of NLR levels and the severity of COVID-19

There is a strong relationship between the increase in NLR levels and the COVID-19 severity based on the Spearman test at a Confidence Interval of 95% and α = 0.05 with a p-value of 0.001 (Table 3). This is shown in the Boxplot Diagram indicating a strong relationship, where the higher the NLR level, the higher the severity of COVID-19 (Figure 1).

DISCUSSION

A number of studies have shown that males are more susceptible to infection than females, and are more hospitalized for Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and COVID-19 infections.[15–17] Several mechanisms that cause males more susceptible to infection with COVID-19 include sex hormone and gene X-related activity which plays a role in modulating the innate and adaptive immune response modulation when infected with the virus.[18]

Besides, the main route of infection with SARS-CoV-2 is through the Angiotensin-converting enzyme 2 (ACE2) receptor, therefore, the biological differences in the receptors greatly affect the susceptibility of disease transmission. A previous study showed that males have higher amounts of ACE2 expression in the circulation and lungs than females.[15] This is in accordance with the results of this study where males were mostly infected with COVID-19 and received treatment in hospital.

Neutrophils are natural immune cells that play a role in the immune system. During the initial infection with pathogenic microorganisms, the cells tend to rapidly converge chemotactically at the infection site and act in the body's defense and immune regulation.[19] Therefore, when the body's

neutrophils significantly reduced, the body's immunity is compromised and the infection risk increases significantly.[20] Lymphocytes are the main effector cells of the human immune response, hence the number of lymphocytes in the body is closely related to the immune and the defense system against pathogenic microorganisms which correlates negatively with the degree of inflammation.[21] Moreover, NLR contains two types of leukocyte subtypes that reflect the body's neutrophil balance, namely lymphocyte count level and degree of systemic inflammation.[22] The NLR reflects a better balance between the inflammatory severity and immune status, therefore, it is considered as an important marker of the systemic inflammatory response.[23]

Based on this, researchers speculated that severe COVID-19 infection causes significant systemic inflammation and NLR plays a role in reflecting the infection severity. Clinical observations showed that some patients with mild disease developed into severe disease with a high mortality risk within a short period of time. This sudden disease severity is due to the rapid onset of acute respiratory distress syndrome (ARDS) and subsequent multi-organ dysfunction that associated with a "cytokine release storm".[7]

In 2003, infection with the SARS-CoV virus was also found to cause ARDS and multiple organ failure, resulting to a very high mortality rate. The fundamental pathology of this event is the discovery of a persistent inflammation storm.[24] Meanwhile, SARS-CoV-2 virus is very similar to the SARS-CoV virus as it belongs to the -CoV coronavirus family.[25]

Based on the close similarity between the two viruses, the clinical condition of a COVID-19 patient that changes from mild to critical is due to a storm of inflammatory factors.[25] Imran et al. showed that NLR was an independent risk factor for severe COVID-19 pneumonia in a severe group.[7] Furthermore, Li et al. showed that some of the proinflammatory cytokines such as interleukin (IL)-2, IL-7, IL-10, granulocyte colony-stimulating factor (GSCF), interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1 alpha (MIP1A) and tumor necrosis factor (TNF) had elevated plasma in patients with severe COVID-19 pneumonia.[26] This certainly occurs due to the inflammatory response in the patient's body, which is in accordance with the results of this study.

Briefly, this study performed a retrospective analysis of common clinical parameters that are easily obtained from the laboratory. This study showed that there was a significant relationship between NLR and the COVID-19 severity. The severe group tended to have a significant higher NLR.

This study has certain limitations, such as being carried out in only one central place. For more accurate, precise and a broader generalization of results, the study needs to be conducted in several places to provide better validation of the results. The authors are grateful to the entire medical team involved in collecting data for this study. There is no conflict of interest in this study.

CONCLUSION

NLR is applicable as an early warning signal for the severity of COVID-19 infection and also provides an objective basis for early detection and management of severe COVID-19 pneumonia. This marker is very important, especially in remote areas where diagnostic tests are limited, which often creates difficulties in diagnosing COVID-19.

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