

Association of bacterial/viral infections with neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio, and platelet-lymphocyte ratio in patients presenting with fever

Tri Yulia Rini¹, Satriawan Abadi¹, Sudirman Katu¹, Syakib Bakri¹, Haerani Rasyid¹, Hasyim Kasim¹, Andi Fachruddin¹, Risna Halim¹, Arifin Seweng²

^{1,2,3,4,5,6,7,8}Department of Internal Medicine, Faculty of Medicine, Universitas Hasanuddin, Makassar 902445, Indonesia

⁹Departement of Biostatistics, Faculty of Public Health, Universitas Hasanuddin 90245, Indonesia

Corresponding author

email: triyuliarini.marissangan@gmail.com

Abstract: Background of the Study: Bacterial and Viral infections are often hard to be distinguished in daily clinical practice. Biological markers obtained from a routine examination play an important role to minimize time in providing diagnose and giving therapy. Recently, the use of Neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR) is greatly considered to differentiate types of infection found in the patients presenting with fever.

Method: This study uses prospective cohort study design and involves patients presenting with fever who are admitted to the ICU. The initial NLR, MLR, and PLR is examined and categorized into types of infection found. The ANOVA test and t-test are performed to find out the difference among study groups with the value of $\alpha = 0,05$.

Result : This study involves 207 patients (92 male patients [44%]) presenting with fever with the average age of $45,6 \pm 14,6$ years old. The majority of cases (135 cases [66,5%]) in patients with fever results from bacterial infection; The study also finds 47 cases (34,8%) of typhoid fever and 30 cases (22,2%) of pulmonary tuberculosis. Dengue hemorrhagic fever (DHF) is the most commonly found viral infection with 52 cases (76,4%). The significant diagnose of bacterial infection shows higher value of NLR and MLR than that of viral infection ($P < 0,001$); Urinary tract infection has the highest value of NLR and MLR, amounting to $9,4 \pm 3,6$ and $0,23 \pm 0,20$, respectively. In general, the value of PLR is lower than that of viral infection ($P < 0,001$).

Conclusion: Neutrophil-lymphocyte ratio, MLR and PLR have benefit to predict diagnosis for the patients presenting with a fever. Bacterial infection is associated with the high value of NLR and MLR, and PLR generally has a lower value in viral infection cases.

Key Words: neutrophil, lymphocyte, monocyte, platelet, fever, bacteria, virus

1. INTRODUCTION

Patients with infection admitted to the ER need immediate and accurate treatment to prevent lethal or dangerous outcome. Some biological markers have been examined in a clinical setting, but the examination has not met a satisfactory result. A simple parameter obtained from complete blood count has now been considered as a potential biological marker in providing diagnosis for a patient admitted to the ER; however, no consensus has been agreed upon its accuracy and significant clinical benefit.¹ Neutrophil-lymphocyte ration, MLR, and the least commonly used, PLR

are of simple parameters which can be considered in treating suspected patients with infection.^{1,2,3}

Neutrophil, lymphocyte, monocyte, platelet are components which directly involves in inflammation process due to bacterial and viral infections.⁴ The three cells serve as the important elements in the body's immunity; moreover, they support the defense system by maintaining hemostasis.^{5,6} In addition, fever is frequently considered as a marker of infection and some inflammation diseases. These symptoms result from the activities of immune cells in which those three inflammation components take a part. Consequently, some visible changes of those components occur in infectious condition, mostly causing fever when patients are admitted to the ER^{2,3}. The most common finding, although it does not always happen, is that bacterial infection is associated with leukocytosis while viral infection is commonly associated with lymphocytosis.²

In this study, we investigate the condition of NLR, MLR, and PLR in the patients with infection who are admitted to the ER.

2. METHOD

Study Design

This observational study uses prospective cohort study design. It was conducted at the Emergency Room in Dr. Wahidin Sudirohusodo Central General Hospital Makassar, Indonesia.

Study Subjects

This study involves adult patients (age > 18 years old) presenting with fever who come to the ER, Dr. Wahidin Sudirohusodo Central General Hospital. These patients will be excluded under following conditions: (a) they have a prior history tumor or malignancies; (b) they pass away when having complete blood count; (c) they are undergoing chemotherapy.

Data Collection

This observational study uses data obtained from the characteristics of study subjects, such as age and sex, clinical symptoms when admitted to the ER, and some parameters during a complete blood count, some of which are neutrophil, lymphocyte, monocyte, and platelet. Study data are compiled by using Microsoft Excel 2010 software.

Study Data Analysis

This study applies statistical tests such as ANOVA test and t-test. The differences among study group is significant if $p < 0,05$. Statistical analysis is performed by using SPSS 25 software (Chicago, Illinois, USA).

Ethical License

The Ethical Commission, Faculty of Medicine, Universitas Hasanuddin has approved all procedures of ethical license and eligibility in performing study using data from human subjects.

3. RESULT

This study involves 207 ER patients presenting with fever as a main symptom. There are 92 male respondents (44%). The average age of the patients is $45,6 \pm 14,6$ years old. In general, the level of leukocytes of all respondents has a normal range (**Table 1**).

Most diagnosis (135/207 [65,2%]) for ER patients presenting with fever refer to suspected or confirmed bacterial infection (**Table 2**).

Correlation between final diagnosis and NLR

Urinary tract infection has the highest NLR mean ($9,4 \pm 3,6$), and acute cholecystitis has the second highest mean ($8,5 \pm 5,0$), then followed by pneumonia ($7,9 \pm 5,0$). Dengue hemorrhagic fever (DHF) has the lowest mean with $1,7 \pm 0,5$. NLR mean and final diagnosis have a significant difference ($P < 0,001$; **Table 3**).

Correlation between final diagnosis and MLR

Urinary tract infection has the highest MLR mean ($0,23 \pm 0,20$), and acute cholecystitis has the second highest mean ($0,22 \pm 0,20$), then followed by pneumonia ($0,21 \pm 0,16$). Pulmonary tuberculosis and dengue hemorrhagic fever (DHF) have the lowest mean with $0,06 \pm 0,03$. There is a significant difference between MLR mean and final diagnosis ($P < 0,001$; **Table 4**).

Correlation between final diagnosis and PLR

Dengue Haemorrhagic Fever and hepatitis B have the lowest PLR mean $78,1 \pm 44,2$ and $72,3 \pm 40,4$, respectively. However, it needs to point out that the sample size of patients with hepatitis B is small (2,4%). There is a significant difference between PLR mean and final diagnosis ($P < 0,001$; **Table 5**).

4. DISCUSSION

Correlation between final diagnosis and NLR

Patients with fever sometimes receives adequate treatment quite late. Forget et al have reported that a normal level of NLR in the healthy population ranges from 0.78 to 3.53, however, controversy on the range of normal level of NLR still occurs due to the inadequate number of studies.⁷ This study has found out that NLR level increase on some diseases commonly caused by bacterial infection, such as urinary tract infection, acute cholecystitis,

pneumonia, diabetic ulcer and pulmonary tuberculosis (**Table 2**; $P < 0,001$). Russell CD et al found out that NLR can be treated as a biological marker for some bacterial infections, for example respiratory tract infection, community-acquired pneumonia, urinary tract infection, diabetic ulcers, pulmonary tuberculosis, and bacterial tonsillitis.⁸

Neutrophil-lymphocyte Ratio is perceived to have a discriminatory level to predict bacteremia if it is compared to provide prediction by involving neutrophilia or lymphocytopenia only.^{7,9} The physiological immune response as the onset of infection and stress is characterized by an increase in neutrophil count and a decrease in leukocyte count. An increase in neutrophil count results from a decrease rate of neutrophil apoptosis and a rapid mobilization of neutrophil from the bone marrow. The lymphocyte count decreases as the result of the migration of activated lymphocytes to the inflammatory tissues and an increase of lymphocyte apoptosis.¹⁰ Consequently, NLR level in the urinary tract infection and acute cholecystitis due to bacterial infection is high.

The inflammatory response plays an important role in the development and progression of pneumonia. The biological markers for inflammation such as CRP, interleukin (IL-), and procalcitonin are able to improve the diagnosis accuracy, but the use of these markers needs astronomical cost. Therefore, these markers are not frequently used. Cataudella E et al has conducted a clinical prospective study which involves 195 elderly patients with community pneumonia, and the result shows that NLR mean of patients with pneumonia is $12,7 \pm 12,9$. Patients with $NLR \geq 11,12$ have an increasing mortality rate and require treatment in the hospital.¹¹ In our study, we also find the same finding, that is, the increasing NLR level on the patients with pneumonia (**Table 2**). In addition to serving as a predictor of mortality and the severity of CAP, NLR can be used to distinguish pulmonary tuberculosis and community pneumonia in the early stage of diagnosis as having been examined in another study. Yoon NB et al in their retrospective observational study on 206 patients suspected with pulmonary tuberculosis and bacterial community pneumonia find out that NLR level on the patients with pulmonary tuberculosis is significantly lower than those with bacterial community pneumonia ($3,67 \pm 2,12$ vs. $14,64 \pm 9,72$, $P < 0,001$).⁹ This study supports the findings of our study in which NLR level on the patients with pneumonia is higher than that on the patients with pulmonary tuberculosis ($7,78 \pm 5,02$ vs. $5,46 \pm 3,85$; **Table 2**).

Similarly, Vatankhah N et al find out that NLR level on diabetic ulcers which have not healed is higher than that on recovered diabetic ulcers ($P = 0,01$).¹² During the inflammation, bacteria invade the body, releases chemokines in vivo, induces cytokine release, stimulate chemotaxis, produce and release a great number of neutrophil in the bone marrow, and trigger local and systemic neutrophils increase. When the series of reaction occur, the immune system of the patients decreases in which a large number of T lymphocytes undergo apoptosis. Apoptosis cells will inhibit most of the activated T lymphocytes and the activity of lymphocytes proliferation also decreases.¹³

This study asserts that some viral infection-caused diseases (hepatitis B, DHR, and HIV infection) show significantly lower NLR level compared to bacterial-infection caused diseases ($P < 0,001$). Holub M et al shows that NLR median value on the patients with

bacterial infection is higher than that of viral infection (2,86 [1,95 – 4,15] vs 1,86 [1,44 – 2,73]). The NLR value of 6,2 is set as the threshold value to distinguish bacterial and viral infections with sensitivity value of 91% and specificity value of 96%.¹⁴ A retrospective study using 52 patients with DHF in Indonesia finds out that the increase of DHF degree has significant correlation with the decrease of NLR level ($P < 0,001$; $r_s = -0,687$).¹⁵ Viral infection induces lymphocyte activation, undifferentiated lymphocyte proliferation, and antibody secretion or cytokines / lymphokines. Immune defense against viral infection depends more on T cells than antibody. Cytotoxic T cells are completely important to eliminate virus-infected cells. Cytotoxic T cells secrete a number of cytokines, such as gamma interferon and tumor necrosis factor (TNF); therefore, leukocyte count and its differentiation provide useful information in differentiating bacterial and viral infections despite having poor sensitivity and specificity. Virus causing hepatitis B and HIV is known to stimulate lymphocytosis leading to a decrease of NLR level.^{16,17}

Leukopenia having a feature of neutropenia and lymphocytosis serves as the relatively common occurrence on the patients with typhoid fever.¹⁸ The finding of the study shows that the mean of leukocytes, neutrophils, and lymphocytes counts in patients with typhoid has a normal range (**Table 2**). This finding is similar to that of a study conducted by Gaffar MSA et al which involves 191 adult patients with typhoid. That study reveals that leukopenia is found in 24,6% of patients; neutropenia is found in 25% of patients, and lymphocytosis was not found.¹⁸ A retrospective observational study involving 75 patients with typhoid fever delineates that most patients have normal result and only 4% of patients have leukopenia. This finding supports a study from Ahmet et al and Rasoollinad et al which explains that leukopenia is noticeable on 18% and 11,2% of patients.¹⁹

Correlation between final diagnosis and MLR

The application of monocyte-lymphocyte ratio as a widely-used biological marker is still quite rare.²⁰ Our study observes that patients suspected to suffer from bacterial infections (UTI, acute cholecystitis, pneumonia, pulmonary tuberculosis, diabetic ulcer, and typhoid fever) have a significantly higher NLR level than those with viral infections or other clinical diagnosis. Djordjevic D et al report that 392 patients who were treated in the intensive unit with critical diseases have higher MLR. Monocyte is an important component in innate immune responses. This component connects the adaptive immune system through the antigen presentation to lymphocyte. Nevertheless, the number of literature discussing the correlation of MLR and some infections is still inadequate.¹

Monocyte can be perceived as target cell of *Mycobacterium tuberculosis*, and lymphocytes are the main effector cells of tuberculosis immunity. In the progression of tuberculosis infection, monocytes perform phagocytose, inhibit mycobacteria, and form granulomas. An increase in MLR indicates that monocytes relatively increases and lymphocytes relatively decreases.²¹ Our study finds out that patients with tuberculosis have MLR of $0,14 \pm 0,11$. This result is lower than that of Wang W et.al, which reports that patients with tuberculosis have significantly higher MLR than that of control group, 0,45 (0,28 – 0,67) and 0,20 (0,17 – 0,25), respectively.²¹ Buttle T et al also reported that 59% of patients with 3+ acid-resistant bacilli examination have an $MLR > 0.45$ and only 33% of patients have an $MLR < 0.45$.²²

This difference in MLR may occur due to the antibiotic which has previously been administered at the secondary level hospital or primary health centre. Thus, it influences the results of the study. Age also influences the function of monocytes. As people grow older, the function of monocytes also decreases.²³ The average age of patients involving in this study is older compared to the age of participants in other study.

Correlation between final diagnosis and PLR

Patients with DHF and hepatitis B have lower PLR than those with other diagnosis. It happens because platelets count in patients with DHF decreases. Platelet of the dengue virus-infected patients experiences mitochondrial dysfunction leading to the activation of apoptosis cascade which results in the cell death. Prolonged thrombocytopenia are more frequently noticeable in DHF case than in dengue fever.²⁴ Our study presents a similar result as a retrospective study from Irmayanti et.al, which reports that all patients with DHF have low platelet count and normal lymphocytes,¹⁵ It implies that PLR in patients with DHF is lower than in healthy adults

The findings of our study show that PLR count in patients with hepatitis B is $72,3 \pm 40,4$; with mean of platelet $153,6 \pm 90,1 \times 10^3/\mu\text{L}$ and lymphocyte $2,2 \pm 0,3 \times 10^3/\mu\text{L}$. A study from Zhao Z et al which compares 172 patients with hepatitis B and 40 healthy adults also present similar findings. Their study shows that patients with hepatitis B have lower PLR count than healthy adults ($72,1 - 181,0$ vs. $204,4$, masing-masing). Chronic hepatitis B has long been known as the cause of thrombocytopenia, even without cirrhosis. However, a number of studies still present different results due to some reasons.²⁵

This study has some weaknesses. First, it has smaller size of study samples. Therefore, it is not adequate to present broader description on some final diagnosis, especially in hepatitis B case. Further, the result has a bias in the interpretation on data used for the study. Second, this study is not able to rule out the co-infection influence in NLR, MLR, and PLR values which accompany the main diagnosis. Thus, the values of those parameters do not represent the real value of a diagnosis..

5. CONCLUSION

This study shows that patients presenting with fever due to bacterial infection have significant correlation with higher NLR value compared to that in viral infection. MLR level found in bacterial infection is higher than that in viral infection. In general, PLR count is lower in cases with viral infection.

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Table 1. Characteristics of Study Subjects

VARIABLES	RESULT
Age (mean \pm SD)	45,7 \pm 14,7
Sex n(%)	
Male	92 (44)
Leukocytes (mean \pm SD)	9,2 \pm 5,1
Neutrophils (mean \pm SD)	6,8 \pm 4,7
Lymphocytes (mean \pm SD)	2,0 \pm 1,1
Monocyte (mean \pm SD)	0,2 \pm 0,2
Platelets (mean \pm SD)	235,7 \pm 103,2

SD, standard deviation

Table 2. Frequency of final diagnosis of patients presenting with fever

FINAL DIAGNOSIS	n(%)
<i>Suspected/Confirmed with bacterial infection</i>	135 (66,5)
Typhoid fever	47 (34,8)

	Pulmonary tuberculosis	30 (22,2)
	Urinary tract infection	5 (3,7)
	Acute cholecystitis	7 (5,1)
	Infected diabetic ulcers	6 (4,4)
	Pneumonia	40 (29,6)
	<i>Suspected/Confirmed with viral infection</i>	68 (33,5)
	Hepatitis B	5 (7,3)
	HIV Infection	11 (16,1)
	DHF	52 (76,4)

Table 3. Mean of NLR based on Final Diagnosis

FINAL DIAGNOSIS	NLR (mean ± SD)	P
Hepatitis B	1,6 ± 0,5	< 0,001
DHF	1,7 ± 0,5	
Typhoid fever	2,3 ± 1,5	
HIV Infection	2,6 ± 1,0	
Pulmonary tuberculosis	5,5 ± 3,9	
Infected diabetic ulcers	5,7 ± 3,7	
Pneumonia	7,9 ± 5,0	
Acute cholecystitis	8,5 ± 5,0	
Urinary tract infection	9,4 ± 3,6	

SD, standard deviation

Table 4. Mean of MNL based on Final Diagnosis

FINAL DIAGNOSIS	MNL (mean ± SD)	P
DHF	0,06 ± 0,03	< 0,001
Pulmonary tuberculosis	0,06 ± 0,03	
Hepatitis B	0,06 ± 0,05	
HIV Infection	0,09 ± 0,04	
Typhoid fever	0,09 ± 0,06	
Infected diabetic ulcers	0,16 ± 0,11	
Pneumonia	0,21 ± 0,16	
Acute cholecystitis	0,22 ± 0,20	
Urinary tract infection	0,23 ± 0,20	

SD, standard deviation

Table 5. Mean of PLR based on Final Diagnosis

INITIAL DIAGNOSIS	PLR (mean ± SD)	P
Hepatitis B	72,3 ± 40,4	< 0,001
DHF	78,1 ± 44,2	

Infected diabetic ulcers	108,7 ± 26,4	
Typhoid fever	148,4 ± 73,1	
Urinary tract infection	173,2 ± 91,5	
Pneumonia	175,5 ± 110,5	
Pulmonary tuberculosis	183,2 ± 116,1	
HIV Infection	225,0 ± 92,6	
Acute cholecystitis	229,1 ± 103,8	

SD, standard deviation