

# INDONESIAN JOURNAL OF CLINICAL PATHOLOGY AND MEDICAL LABORATORY

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

---

## CONTENTS

### RESEARCH

Differences of Plasma Interleukin-6 and Tumor Necrosis Factor- $\alpha$ Levels in Healthy People, Rifampicin Resistant and Sensitive Pulmonary Tuberculosis Patients <b>Wahyu Setiani Wibowo, Jusak Nugraha, Soedarsono</b>	129 - 134
Association between Specific Enolase Serum Levels and Outcome Acute Ischemic Stroke One Month After Onset <b>Yuri Haiga, Darwin Amir, Yuliarni Syafrita</b>	135 - 139
Analysis of Hemoglobin Levels And Leukocyte Count in Neonates with Hyperbilirubinemia <b>Dewi Suharti, Sulina Yanti Wibawa, Muthmainnah</b>	140 - 144
Diagnostic Value of Ca-125 in Patients with Epithelial Ovarian Cancer at the Dr. Soetomo General Hospital Surabaya in 2016 <b>Kintan P. R. Kania, Betty A. Tambunan, Willy Sandhika</b>	145 - 149
Analysis of Vitamin D in Patients with Type 2 Diabetes Mellitus <b>Arfandhy Sanda, Uleng Bahrin, Ruland DN. Pakasi, Andi Makbul Aman</b>	150 - 154
Proportion of Rhesus Blood Phenotypes at the Blood Donor Unit in Bandung City <b>Ivana Dewi, Nadjwa Zamalek Dalimoenthe, Anna Tjandrawati, Nida Suraya</b>	155 - 160
Correlation of Total Lymphocyte Count with CD4 Count in HIV/TB Coinfected Patients <b>Herniaty Rampo, Uleng Bahrin, Mansyur Arif</b>	161 - 164
Using Six Sigma to Evaluate Analytical Performance of Hematology Analyzer <b>Robiul Fuadi</b>	165 - 169
Correlation of AA Index with Degree of Liver Fibrosis in Chronic Hepatitis B Patients <b>Rika Andriany, Ibrahim Abdul Samad, Mansyur Arif</b>	170 - 173
Difference in HbA1c Level between Boronate Affinity and Ion Exchange-High Performance Liquid Chromatography Method in Diabetic Patient <b>Tuti Asryani, Ellyza Nasrul, Rikarni, Tutty Prihandani</b>	174 - 179
Diagnostic Value of Neutrophil Lymphocyte Ratio to Differentiate Ischemic and Hemorrhagic Stroke <b>Martina Rentauli Sihombing, Liong Boy Kurniawan, Darwati Muhadi</b>	180 - 183
D-Dimer and Fibrinogen in Patients Underwent Surgery in Malignant and Benign Ovarian Tumor <b>Ismail Aswin, Herman Hariman, Fauzie Sahil</b>	184 - 190

Relationship between Specific Gravity of Cupric Sulfate and Saturation of Blood Droplets During Donor's Hemoglobin Screening <b>Resna Hermawati, Solichul Hadi</b> .....	191 - 193
Vancomycin-Resistant <i>Staphylococcus aureus</i> at the Dr. Wahidin Sudirohusodo Hospital Makassar <b>Fatmawaty Ahmad, Nurhayana Sennang, Benny Rusli</b> .....	194 - 198
The Levels of Interleucin-6 (Il-6) and Tumor Necrosis Factor Alpha (TNF-ALFA) in Preeclampsia Patient and Normal Pregnancy <b>Mawardi, Ratna Akbari Ganie, Sarma N. Lumbanraja</b> .....	199 - 201
Analysis of Platelet Volume Mean, Platelet Distribution Width, and Platelet Count in Hemorrhagic and Non-Hemorrhagic Stroke <b>Gita Medita Sunusi, Darwati Muhadi, Mansyur Arif</b> .....	202 - 206
High Fluorescent Lymphocyte Count Examination in Dengue Hemorrhagic Patients with Sysmex Xn-1000 Hematology Analyzer <b>Budiono Raharjo, Solichul Hadi</b> .....	207 - 210
Prevalence and Characteristics of Multidrug-Resistant <i>Acinetobacter baumannii</i> Cases at the Dr. Wahidin Sudirohusodo General Hospital in Makassar <b>Dewi Kartika Tungadi, Nurhayana Sennang, Benny Rusli</b> .....	211 - 217
The Correlation of Anemia and Hepcidin Serum Levels in Regular Hemodialysis Patients with Chronic Hepatitis C <b>Wingsar Indrawanto, Adi Koesoema Aman, Alwi Thamrin</b> .....	218 - 223
The Comparison between HbA1c and Glycated Albumin Level Patient with Type II Diabetes Mellitus with or without CKD <b>M. Rusli, Zulfikar, Santi Syafril</b> .....	224 - 227
Differentiation of $\gamma\delta$ Lymphocyte Cells Expressing Interleukin-17 on Healthy Persons and Adult Acute Myeloid Leukemia Patients <b>Elvan Dwi Widyadi, Yetti Hernaningsih, Endang Retnowati, Ugroseno, Ryzky Widi Atmaja</b> .....	228 - 232

## LITERATURE REVIEW

Hormone Examination in Menopause <b>Ferdy Royland Marpaung, Trieva Verawaty Butarbutar, Sidarti Soehita</b> .....	233 - 239
--	-----------

## CASE REPORT

Chronic Myelogeneous Leukemia Transformation into Acute Lymphoblastic Leukemia <b>Endah Indriastuti, Arifoel Hajat</b> .....	240 - 245
Rapid Progression of Clavicular Solitary Plasmacytoma to Multiple Myeloma <b>Hantoro Gunawan, Paulus Budiono Notopuro</b> .....	246 - 249

## DIAGNOSTIC VALUE OF NEUTROPHIL LYMPHOCYTE RATIO TO DIFFERENTIATE ISCHEMIC AND HEMORRHAGIC STROKE

Martina Rentauli Sihombing<sup>1</sup>, Liong Boy Kurniawan<sup>2</sup>, Darwati Muhadi<sup>2</sup>

1 Medical Doctor Specialist Education Program of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. E-mail: [martina.march@yahoo.com](mailto:martina.march@yahoo.com)

2 Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar

### ABSTRACT

Stroke is a neurologic emergency disease and the main cause of high mortality. The inflammatory process in stroke due to cell and tissue damage causes an increase of leucocyte prominently neutrophil. Neutrophil Lymphocyte Ratio (NLR) is an easy-to-measure inflammatory marker. There is only a few data of NLR in Indonesia. This study aimed to know the difference in NLR values among ischemic and hemorrhagic stroke and to find out the diagnostic NLR to differentiate ischemic and hemorrhagic stroke patients. This study was a retrospective cross-sectional study using secondary data from Medical Record of Wahidin Sudirohusodo Hospital, Makassar. Leucocyte, neutrophil, and lymphocyte first data from adult stroke diagnosed patients were taken. Data statistically analyzed and diagnostic value of NLR was determined by Receiver Operating Curve (ROC) analysis. Total of 402 patients was enrolled, 214 (54.72%) with ischemic stroke and 182 (46.8) with hemorrhagic stroke. There was a significant NLR difference between ischemic stroke (median 7.23) and hemorrhagic stroke (median 3.65) ( $p < 0.001$ ). Using cut-off 5.18, ROC curve showed of (AUC) 0.730 which had a weak performance to differentiate ischemic and hemorrhagic stroke with sensitivity 67.8% and specificity 68.6%. The neutrophil-lymphocyte ratio in hemorrhagic stroke is higher than ischemic stroke. Further studies with larger and more evenly distributed samples and consideration of sampling time are a suggestion.

**Key words:** Stroke, ischemic, hemorrhagic, NLR

### INTRODUCTION

Stroke is defined by the World Health Organization (WHO) as a clinical syndrome consisting of rapidly developing clinical signs of focal (or global in case of coma) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than a vascular origin.<sup>1</sup> Stroke is a serious neurological emergency and ranked high among death causes. Stroke affects approximately 750,000 USA people every year and ranked third among mortality causes. In every 1000 people in Indonesia, there are eight suffer from a stroke in the population of 45-54 years old.<sup>2,3</sup>

Two types of stroke are; hemorrhagic and ischemic stroke. Hemorrhagic strokes are the strokes that caused by the rupture of blood vessels in the brain which can inundate and suppress the brain tissues and causes damages in brain coordination function.<sup>2</sup> The reduced blood flow to an area in brain tissues caused ischemic strokes. Reperfusion injury in ischemic stroke is a further dysfunction of the brain due to inflammation response to body reaction to returning the blood flow to the ischemic area in

stroke. The inflammatory response begins with the production of proinflammatory cytokines in an ischemic area that will result in mobilization and upregulation of leukocytes, particularly the neutrophil.<sup>4</sup>

According to several studies, the extent of damage in brain tissue correlates to the presence of leukocyte accumulation. A study by Grigorian suggests that neutrophil accumulation is first detected in the first six hours after the occurrence, and reaches a maximum at 24 hours and remains at a high level until nine days before it decreases.<sup>5,6</sup> A study by Hamzah suggests that the higher stroke lesion volume will cause higher neutrophil number either in ischemic or hemorrhagic stroke.<sup>7</sup>

Neutrophil-Lymphocyte Ratio (NLR) is an inflammation marker which is currently being used in various studies due to its simple procedure and inexpensive cost compared to other markers such as C-Reactive Protein (CRP) and procalcitonin.<sup>8,9</sup> At the beginning of its process, stroke induces the entrance of neutrophil into the brain but not lymphocyte, making the ratio increases. The neutrophilic reaction caused by the stroke is not significantly high, that the

average value of neutrophil is not highly significant compared to NLR. The physiological NLR is less than 5 (normal neutrophil value <75% and lymphocyte >15%). In a pathologic condition, for example by severe infection or systemic inflammation, the neutrophil-lymphocyte ratio increases to more than 6.<sup>10-12</sup> A study by Gokhan *et al.* showed that NLR patients with hemorrhagic and ischemic stroke increased significantly that it could be used in establishing the diagnosis and as prognostic predictor in hemorrhagic or ischemic stroke.<sup>10</sup>

Data on NLR in stroke in Indonesia, as far as the authors know, is limited. Therefore we are interested in studying the NLR in ischemic and hemorrhagic stroke patients in the Dr. Wahidin Sudirohusodo Hospital, Makassar. This study is expected to help clinicians to differentiate ischemic and hemorrhagic stroke based on the neutrophil-lymphocyte ratio. This study aims to know the difference in NLR values among ischemic and hemorrhagic stroke and to find out the diagnostic NLR to differentiate ischemic and hemorrhagic stroke patients.

## METHODS

This study was a cross-sectional retrospective study using secondary data obtained from the Medical Record Installation of Dr. Wahidin Sudirohusodo Hospital Makassar, from July 2015 to June 2016. From 1289 patients diagnosed with stroke, 402 patients were meeting the inclusion criteria. Inclusion criteria in this study were adult patient (above 18 years old) diagnosed with hemorrhagic or ischemic stroke by a clinician based on evaluation results and CT scan completed with routine blood test results taken during admission to the Dr. Wahidin Sudirohusodo Hospital, Makassar. The neutrophil-lymphocyte ratio was the results of absolute neutrophil value divided by absolute lymphocyte value and expressed as cell/ $\mu$ L. This data

was obtained from routine blood results using K3EDTA blood sample and measured with flow cytometer Sysmex XT-2000i.

The obtained results were analyzed statistically with SPSS software. The data were analyzed using the Kolmogorov-Smirnov test to determine the distribution, and Mann-Whitney to compare NLR values in ischemic and hemorrhagic stroke with not normally distributed data, and the establishment of cut-off value using ROC curve. The results were presented in the form of tables.

## RESULTS AND DISCUSSION

The total number of the samples was 402 with average age  $60.40 \pm 12.01$ , with more dominant male subject (229 [54.7%]) and ischemic stroke as the most common stroke (214, [53.2%]). The mean value of leukocyte was  $12.06 \pm 4.72$ , mean neutrophil  $74.40 \pm 11.94$ , mean lymphocyte  $16.39 \pm 8.72$  and mean NLR  $6.87 \pm 5.6$  (Table 1).

Data were analyzed by the Mann-Whitney test for age, leukocyte, neutrophil, lymphocyte, and neutrophil because the sample distribution was not normal. A significant difference was found in age ( $p < 0.001$ ), leukocyte ( $p < 0.001$ ), neutrophil ( $p < 0.0001$ ), lymphocyte ( $p < 0.001$ ), and NLR ( $p < 0.01$ ) between ischemic stroke and hemorrhagic stroke, as shown in Table 2.

Analysis of NLR Receiver Operating Characteristics (ROC) of study subjects with hemorrhagic and ischemic stroke with CT scan results, the AUC-value was 0.730 with cutting point 5.181; sensitivity 67.8%; specificity 68.6% (Figure 1).

This study showed that the number of male subjects was higher than female subjects and the mean age was  $60.4 \pm 12.01$ . This finding indicated that stroke was more frequently found in older patients. following the study of Alexander that stroke

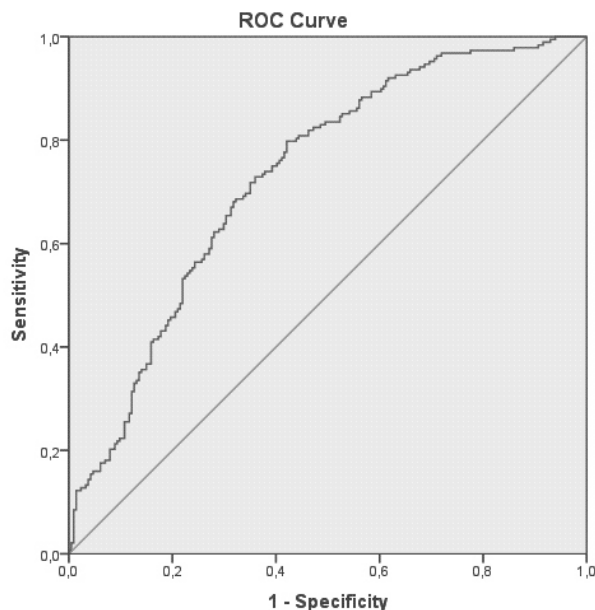
**Table 1.** Characteristics of study subjects

Characteristic	N (%)	Mean $\pm$ SD	Median	Min-Max
Age (years)		$60.40 \pm 12.01$	60	28-95
Gender				
Male	220 (54.7)			
Female	182 (45.3)			
Stroke type				
Ischemic	214 (53.2)			
Hemorrhagic	188 (46.8)			
Leukocyte value ( $10^3/\mu$ L)		$12.06 \pm 4.72$	11.2	4.0-31.50
Neutrophil value (%)		$74.40 \pm 11.94$	76.25	33.70-94.0
Lymphocyte value (%)		$16.39 \pm 8.72$	15.1	1.0-48.70
NLR		$6.87 \pm 5.6$	5.07	0.69-37.33

**Table 2.** Analysis results based on stroke type

	Ischemic stroke			Hemorrhagic stroke			p-value
	Mean $\pm$ SD	Median	Min-Max	Mean $\pm$ SD	Median	Min-Max	
Age (year)	62.22 $\pm$ 11.9	62.0	30-95	58.30 $\pm$ 11.8	57.0	28-92	<0.001*
Leukocyte (10 <sup>3</sup> / $\mu$ L)	10.58 $\pm$ 4.0	9.8	4.0-26.9	13.73 $\pm$ 4.94	12.8	5.5-31.50	<0.001*
Neutrophil value (%)	70.01 $\pm$ 12.2	69.5	33.7-92.9	79.31 $\pm$ 9.45	81.0	52.6-94.0	<0.001*
Lymphocyte value (%)	19.71 $\pm$ 9.03	19.2	0.1-48.7	12.69 $\pm$ 6.61	11.35	2.40-35.90	<0.001*
NLR	5.12 $\pm$ 6.61	3.65	0.69-32.89	8.82 $\pm$ 6.19	7.23	1.51-37.33	<0.001*

\*Mann-Whitney Test, NLR = Neutrophil Lymphocyte Ratio

**Figure 1.** ROC curve of neutrophil/lymphocyte ratio in stroke patients against CT Scan

incidence rate and possible damages resulted from various risk factors will increase more than two-fold after the age of 55 years old.<sup>13</sup>

This study also showed that 214 (53.2%) patients referred to the Dr. Wahidin Sudirohusodo Hospital Makassar with ischemic stroke diagnosis, outnumbering the hemorrhagic stroke (188;[46.8%]). The result was in accordance with O'Donnel *et al.* study. In a multi-center study, they found higher ischemic stroke incidence (78%) compared to hemorrhagic stroke.<sup>14</sup>

The median of leukocyte value in study subjects with hemorrhagic stroke (12.8x10<sup>3</sup>/ $\mu$ L) was higher compared to ischemic stroke (9.8x10<sup>3</sup>/ $\mu$ L). This result was following Hatta *et al.* study that found higher leukocyte increase in hemorrhagic stroke compared to ischemic stroke. Leukocytes in ischemic stroke, after the cell death, are released in the circulation and tissues for only 3-6 hours, and the highest leukocyte migration occurs at 24-72 hours after ischemic onset and then decreases up to the seventh day.<sup>15-17</sup>

Median neutrophil in hemorrhagic stroke (81%) was lower compared to ischemic stroke (69.5%). Conversely, median lymphocyte in hemorrhagic stroke (11.35%) was lower compared to ischemic stroke (19.2%). The type of leukocytes mobilized due to acute inflammation is neutrophil.<sup>18</sup> Proinflammatory cytokines, particularly interleukin-8, play a role in recruiting neutrophil as an inflammatory response. Reperfusion injury occurs due to neutrophil buildup and its inflammation in ischemic stroke lesion area.<sup>4</sup>

Proinflammatory blood cells in hemorrhagic stroke including neutrophils are released directly in brain tissues within less than 48 hours. The released thrombin due to hemolysis is the potential stimuli for the inflammation to occur in hemorrhagic stroke. Reperfusion injury is not observed in hemorrhagic stroke.<sup>4,15</sup> Decreased lymphocyte level is more due to lymphocyte activities, lymphocyte adhesion to endothelial cells increases during inflammation followed by lymphocyte migration to tissues having inflammation.<sup>17,18</sup>

Study findings indicated a significant difference in NLR values in ischemic and hemorrhagic stroke. The diagnostic test for NLR showed the cutting point at 5.181. Receiver operating characteristics analysis revealed AUC = 0.73, indicating weak AUC-value, with a sensitivity of 67.8% and specificity 68.6%. When the NLR in routine blood is higher than cut-off value, it can be concluded as hemorrhagic stroke, and conversely, when the NLR value is lower than cut-off value, it is an ischemic stroke.

The limitations of this study included the retrospective method from secondary data, medical record data. Other causes of infection or inflammation could not be ruled out entirely. Another limitation was the different onset of checked routine blood after a stroke attack.

## CONCLUSIONS AND SUGGESTION

Study results indicate that NLR value in hemorrhagic stroke is higher than ischemic stroke.

Further studies are suggested with cohort method and larger samples and normally distributed by considering the underlying diseases that can result in bias and considering the time of routine blood collection which represent the peak time for the neutrophil to enter circulation.

## REFERENCES

1. World Health Organization. Stroke, cerebrovascular accident. (Cited on 2017 June, 1<sup>st</sup>). Available at [www.who.int/topics/cerebrovascular\\_accident](http://www.who.int/topics/cerebrovascular_accident).
2. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, *et al*. On behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics 2017 update: A report from the American Heart Association. *Circulation*. 2017; 135: e229
3. Departemen Kesehatan Indonesia. 8 orang dari 1000 orang di Indonesia terkena stroke. (Cited on 2016 June, 1<sup>st</sup>). Available at [www.depkes.go.id](http://www.depkes.go.id).
4. Clark W. Reperfusion injury. (Cited on 2016 June 3<sup>rd</sup>). Available at [www.emedicine.com/neuro](http://www.emedicine.com/neuro).
5. Bonaventura A, Liberale L, Vecchie A, Casula M, Carbone F, *et al*. Update on inflammatory biomarkers and treatments in ischemic stroke. *International Journal of Molecular Sciences*. 2016; 17: 1967.
6. Chen J, Hu X, Stenzel-Poore M, Zhang JH. Immunological mechanism and therapies in brain injuries and stroke. New York, Springer, 2014; 223-35.
7. Hamzah SRM. Leucocyte count in the ischemic and hemorrhagic stroke patient. *Journal Majority*. 2015; 4(1): 86-93.
8. Zhang J, Ren Q, Song Y, He M, Zeng Y, *et al*. Prognostic role of the neutrophil-lymphocyte ratio in a patient with acute ischemic stroke. *Medicine Journal (Baltimore)*. 2017; 96(45): e8624.
9. San I, Icme F, Yuzbasioglu Y, Otal Y, Coskun S, Sener A, *et al*. The prognostic relationship between complete blood count parameter and infarct positive transient ischemic attack. Infarct negative transient ischemic attack and ischemic stroke. *Acta Medica Mediterranea* 2016; 32:97.
10. Gokhan S, Ozhansenekler A, Durgun M, Akil E, Ustundag M, Orak M. Neutrophil-lymphocyte ratio in stroke subtypes and transient ischemic attack. *European Review for Medical and Pharmacological Sciences*. 2013; 17: 653-57.
11. Brooks SD, Spears C, Cummings C, VanGilder RL, Stinehart KR, *et al*. Admission neutrophil-lymphocyte ratio predicts 90-day outcome after endovascular stroke therapy. *J Neurointerv Surg*, 2014 6: 578-83.
12. Rukshin M, Jessani N, Medina Miriam, Blaha J, Rukshin V. The diagnostic value of the neutrophil-lymphocyte ratio in stroke recognition. *The Columbia Science Journal*, 2016; 251(6273): 571.
13. Alexander LL. Ischemic stroke: CME resource. Sacramento California. 2011. Available at <http://www.netce.com/coursecontent.php?courseid=1485>.
14. O'Donnel MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, *et al*. Risk factor for ischemic and intracerebral hemorrhage stroke in 22 countries (the interstroke study); A case-control study. *Population Health Research Institute. Canada, Lancet*, 2010; 10; 376(9735): 112-23
15. Agnihotri S, Czap A, Staff I, Fortunato G, McCullough L. Peripheral leukocyte counts and outcomes after intracerebral hemorrhagic. *Journal of Neuroinflammation*. 2011; 8: 160-4.
16. Hatta S, Ilyas M, Murtala B, Liyadi F. Profil hitung leukosit darah pada fase akut stroke hemoragik dan iskemik dihubungkan volume lesi pada pemeriksaan CT Scan kepala. *Medica Nusantara*, 2010; 25: 1-6.
17. Bain BJ, Bates M, Laffan MA. Reference ranges and normal values. *Dacie and Lewis practical hematology*. 12<sup>th</sup> Ed., Philadelphia. Churchill Livingstone, 2016; 8-17.
18. Hoffbrand A, Petit J. *Essential hematology*. 7<sup>th</sup> Ed., Jakarta, EGC. 2016; 102-13.