



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

CORRELATION BETWEEN NEUTROPHIL-TO-LYMPHOCYTE RATIO AND HIGH SENSITIVITY C REACTIVE PROTEIN WITH SEVERITY AND OUTCOME IN ACUTE ISCHEMIC STROKE PATIENT

Heru Pranata*¹, Puji Pinta O. Sinurat² & Chairil Amin Batubara²

*¹Resident, Department of Neurology, Universitas Sumatera Utara

²Staff, Department of Neurology, Universitas Sumatera Utara

DOI: <https://doi.org/10.29121/ijrsm.v7.i8.2020.11>

Keywords: ischemic stroke, Neutrophil-to-Lymphocyte ratio, High Sensitivity C Reactive Protein, severity, outcome.

Abstract

Background: Inflammation plays a role in the pathophysiology of cerebral ischemic and also an initial response to brain injury. Inflammation involving Blood and Brain Barrier disturbance, leukocyte infiltration, endothelial cells activation, oxidant and inflammatory mediator buildups which can develop rapidly within hours and can cause secondary injury to brain tissues

Objectives: To determine the correlation between Neutrophil-to-Lymphocyte Ratio (NLR) and High Sensitivity C Reactive Protein (hsCRP) with severity and outcome in acute ischemic stroke patients

Methods: This study used a cross-sectional design approach. Sampling was done at H. Adam Malik General Hospital Medan and taken as many as 38 subjects. NLR and hsCRP were examined on the first day of admission. Severity was assessed by using the National Institutes of Health Stroke Scale and the outcome was assessed by using the Modified Ranking Scale on the first and fourteenth day of admission. The data analysis used the contingency coefficient correlation test and gamma test.

Results: Demographic characteristics of the subjects are; the average age is 62 years old, the highest educational level is high school graduate, and the most occupation is unemployed. There is a positive, moderate, and significant correlation between NLR and severity of acute ischemic stroke ($r=0.511$; $p=0.001$). There is a positive, moderate, and significant correlation between NLR and the outcome of acute ischemic stroke ($r=0.463$; $p=0.001$). There is a positive, very strong, and significant correlation between hsCRP and severity of acute ischemic stroke ($r=0.896$; $p=0.001$). There is a positive, strong, and significant correlation between hsCRP and outcome of acute ischemic stroke ($r=0.624$; $p=0.001$)

Conclusions: There is a positive and significant correlation between NLR and hsCRP with severity and outcome in acute ischemic stroke patients.

Introduction

Many factors play a role and involved in stroke occurrence, one of those factors is the inflammatory process. Inflammation plays a role in the pathophysiology of cerebral ischemic and also an initial response to brain injury.¹ The inflammatory process involving Blood and Brain Barrier disturbance, leukocyte infiltration, endothelial cells activation, oxidant and inflammatory mediator buildups which can develop rapidly within hours and can cause secondary injury to brain tissues.² Ischemic tissues will release chemokines and cytokines and then take leukocytes from the peripheral circulation. Among leukocyte cells, neutrophils are found to be an important mediator and trigger ischemic injury in the brain. Neutrophils are identified that it is associated with the extension of infarct volume and increasing stroke severity. It was also mentioned that lymphocytes infiltrate ischemic tissues and mediate inflammatory responses.³

At present, Neutrophil-to-Lymphocytes Ratio (NLR) is often used as a marker to predict outcome in ischemic stroke patients, NLR is considered as a marker that can be evaluated together with negative effects which arising from increased neutrophils as an indicator of acute inflammation and also decreased lymphocytes from physiological stress signal.⁴ Xue et al (2017) mentioned the increase of white blood cells on the early phase of acute ischemic stroke especially neutrophils shows that it correlates with severity in stroke cases.⁵

In brain ischemia, acute local inflammatory processes and changes in inflammation cytokines have occurred, one of which is C Reactive Protein (CRP). C Reactive Protein (CRP) is a blood protein that synthesized in the liver and increased due to inflammatory response which also a biomarker of inflammation in cardiovascular and ischemic stroke cases.⁶ Pandev et al (2018) mentioned the inflammation of blood vessels is related to CRP,



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

increasing of CRP levels can cause injury of brain tissue to become more severe and widespread, so it can cause the outcome in patients to worsen.⁷ High Sensitivity C Reactive Protein (hsCRP) can measure a very low CRP level in the blood, so it is more sensitive in the measurement range 0.1-20mg/L and can be used to detect a low-grade inflammation and it is also a sensitive marker of inflammation occurrence and blood vessel wall injuries.^{8,9}

Method

Study samples

The study samples were taken from patients with acute ischemic stroke in Adam Malik General Hospital with a consecutive sampling technique. The study subjects consisted of 38 first ever stroke patients and were willing to participate in this study by signing a research-informed consent sheet.

Study design

This study used a cross-sectional design approach without treatment interventions. Patients who have been diagnosed with first ever ischemic stroke were examined for NLR and hsCRP on the first day of the admission. Then the stroke severity was assessed by calculating the National Institutes of Health Stroke Scale (NIHSS) score and the outcome was assessed by calculating the Modified Ranking Scale (mRS) score on the first and fourteenth day of admission.

Statistical analysis

Data from the study were analyzed statistically using the SPSS Windows computer program (Statistical Product and Science Service) version 25.0 to analyze the correlation between study variables, in this case, to determine the correlation between NLR and hsCRP with severity and outcome in ischemic stroke patients by using contingency coefficient correlation test and gamma test.

Results

Acute ischemic stroke patient who received treatment at H. Adam Malik General Hospital Medan in January until June 2020, there were 38 subjects with acute ischemic stroke who participated in this study.

Based on the characteristics of 38 study subjects, the mean age of the study subjects is 62 years old \pm 12.43 divided into 3 age range. The age range 36-<51 years old as many as 6 subjects (11.28%), age range 51-<66 years old as many as 18 subjects (35.3%), age range 66-<81 years old as many as 9 subjects (17.6%) and the oldest is \geq 81 years old as many as 5 subjects (9.8%). The most gender of the subjects is a woman as many as 21 subjects (55.3%). The most educational level of the subjects is high school graduates as many as 12 subjects, and the fewest is unschooled as many as 3 subjects (7.9%). The most occupation of the subjects is unemployed as many as 12 subjects (31.6%) and the fewest is an entrepreneur as many as 3 subjects (7.9%). For complete data about the characteristics of the study, subjects are shown in table 1 below.

Table 1. Demographic Characteristics of Study Subjects (n = 38)

Characteristics	Frequency n=38	Percentage (%)
Age, average \pm SD (years)	62 \pm 12.43	
- 36-<51 years old	6	11.8
- 51-<66 years old	18	35.3
- 66-<81 years old	9	17.6
- \geq 81 years old	5	9.8
Sex		
- Male	17	44.7
- Female	21	55.3
Educational Level		
- Unschooled	3	7.9
- Elementary school	5	13.2
- Middle School	8	21.1
- High School	12	26.3
- University	10	26.3



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Occupation		
- Unemployed	12	31.6
- Civil servant	4	10.5
- Entrepreneur	5	13.2
- Private employee	3	7.9
- Farmer	4	10.5
- Retired	10	26.3

In the subject group with $NLR > 5$, there is 1 subject who has a mild level of severity (9.1%), 9 subjects have a moderate level of severity (81.8%) and 1 subject has a severe level of severity (9.1%). While in the subject group with $NLR \leq 5$, 20 subjects have a mild level of severity (74.1%), 6 subjects have a moderate level of severity (22.2%), and 1 subject has a severe level of severity (3.7%). A contingency coefficient test was performed on 38 study subjects, a significant correlation was found between the Neutrophil-to-Lymphocyte Ratio and severity of acute ischemic stroke with p-value 0.001 ($p < 0.05$), Those have a positive and moderate correlation, with r value = 0.511. This can be seen in Table 2 below.

Table 2. Correlation Between NLR and Stroke Severity

NLR	Severity Levels						R	p-value
	Severe	%	Moderate	%	Mild	%		
>5	1	9.1	9	81.8	1	9.1	0.511	0.001
≤ 5	1	3.7	6	22.2	20	74.1		

In the subject group with $NLR > 5$, there is 1 subject who has a good outcome (9.1%) and 10 subjects have poor outcomes (90.9%). While in the subject group with $NLR < 5$, 18 subjects have good outcomes (66.7%) and 9 subjects have poor outcomes (33.3%). A correlation test was performed with the contingency coefficient test on 38 study subjects, a significant correlation was found between Neutrophil-to-Lymphocyte Ratio and the outcome of acute ischemic stroke with p-value 0.001 ($p < 0.05$). Those have a positive and moderate correlation, with r value = 0.463. This can be seen in Table 3 below.

Table 3. Correlation Between NLR and Stroke Outcome

NLR	Outcomes				R	p-value
	Poor	%	Good	%		
>5	10	90.9	1	9.1	0.463	0.001
≤ 5	9	33.3	18	66.7		

In the subject group with a high hsCRP level, 7 subjects have a moderate level of severity (100%) and no subject has a mild and severe level of severity (0%). While in the subject group with a moderate hsCRP level, 2 subjects have a severe level of severity (16.7%), 7 subjects have a moderate level of severity (58.3%), and 3 subjects have a mild level of severity (25%). In the subject group with low hsCRP level, no subject has a severe level of severity (0%), 1 subject has a moderate level of severity (5.3%), and 18 subjects have a mild level of severity (94.7%). Based on the correlation test with the gamma test on 38 study subjects, it was found that there is a significant correlation between hsCRP and severity of acute ischemic stroke with p-value 0.001 ($p < 0.05$). Those have a positive and very strong correlation, with r value= 0.896. This can be seen in Table 4 below.

Table 4. Correlation Between hsCRP and Stroke Severity

hsCRP	Severity Levels						R	p-value
	Severe	%	Moderate	%	Mild	%		
High	0	0	7	100	0	0	0.896	0.001
Moderate	2	16.7	7	58.3	3	25		
Low	0	0	1	5.3	18	94.7		



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

In the subject group with a high hsCRP level, 7 subjects have poor outcomes (100%) and no subject has a good outcome (0%). While in the subject group with a moderate hsCRP level, 10 subjects have poor outcomes (83.3%) dan 2 subjects have good outcomes (16.7). In the subject group with low hsCRP level, 2 subjects have poor outcomes (10.5%) and 17 subjects have good outcomes (89.5%). Based on the contingency coefficient test conducted on 38 study subjects, the results showed that there is a significant correlation between hsCRP and outcome of acute ischemic stroke with p-value 0.001 ($p < 0.05$). Those have a positive and moderate correlation, with r value = 0.624. This can be seen in Table 5 below.

Table 5. Correlation Between hsCRP and Stroke Outcome

hsCRP	Outcomes				R	p-value
	Poor	%	Good	%		
High	7	100	0	0	0.624	0.001
Moderate	10	83.3	2	16.7		
Low	2	10.5	17	89.5		

Discussions

The average age characteristics of acute ischemic stroke patients in this study are relevant to the previous study which conducted by Rambe et al (2013), it mentioned that the average age of acute ischemic stroke patients is 59 years old (age range 20-95 years old).¹⁰ In general, stroke is a disease in the elderly, the prevalence of stroke is found increases with age. The risk of stroke has doubled every decade after the age of 55.¹¹ Reilly and McCullough (2018) mentioned that older stroke patients have a higher mortality rate compared to younger ages.¹² The most sex characteristics of acute ischemic stroke patients in this study are women, as many as 21 subjects (55.3%), this is relevant to the previous study by Rambe et al (2013) which found that there were more woman stroke patients (52.7%) than men (47.3%).¹⁰ Women have several different biological factors than men, including coagulation factors, hormonal reproductive factors, pregnancy and childbirth, and social factors that can influence the stroke risk and the outcome of stroke.¹³ The most occupational characteristics of acute ischemic stroke patients in this study are unemployed as many as 12 subjects (31.6%) and the least are private employees as many as 3 subjects (7.9%). The prevalence of stroke is higher in unemployed people based on the diagnosis of health services or symptoms by 18%.¹⁴ The most educational level characteristics of the study subjects are high school graduates, as many as 12 subjects (12.5%). The prevalence of stroke tends to be higher in people with low educational level based on the diagnosis of health services or symptoms by 32.8%.¹⁴

In this study, there is a significant correlation between NLR and severity of acute ischemic stroke ($r = 0.511$; $p = 0.001$). It is relevant to a previous study conducted by Switonska et al (2019), that an increased NLR can indicate increased severity of acute ischemic stroke ($r = 0.5$; $p = 0.00001$).¹⁵ A study conducted by Hunaifi and Cahyawati (2019) in 52 acute ischemic stroke patients also stated that NLR is associated with increased severity of acute ischemic stroke. The higher the level of NLR can cause the extent of cerebral infarction and increasing severity level ($r = 0.351$; $p = 0.023$).¹⁶ In this study, there is a significant correlation between RNL and the outcome of acute ischemic stroke ($r = 0.463$; $p = 0.001$). There have been several previous studies that also studied the correlation between NLR and outcome of acute ischemic stroke, those studies found that increased RNL is associated with worsening outcome in patients with acute ischemic stroke.⁴ Yu et al (2018) also got a similar result, an increased RNL is associated with poor outcome in patients with acute ischemic stroke ($p = 0.0002$).¹⁷

This is because the inflammatory response itself has an important role in brain tissue damage in ischemic stroke cases. The brain responds to ischemic processes through the activation of white blood cells accompanied by the production of proinflammatory mediators that cause infiltration of various inflammatory cells (neutrophils, lymphocytes) into the brain tissue. In the acute phase, the proinflammatory mediator is released by ischemic brain tissues, which stimulates the expression of adhesion molecules in endothelial blood vessels and leukocytes. This process causes adhesion of leukocytes to the endothelium, followed by the process of migrating leukocytes out of the blood vessels. In the subacute phase, the leukocytes release cytokines and chemokines that strengthen inflammatory reactions and cause disruption of the blood-brain barrier, edema, and neuronal death.¹⁸ The increase of neutrophil count after stroke is due to increased production, increased release of bone marrow and spleen, and decreased neutrophil apoptosis. While lymphocytes number decreased after ischemic stroke. In patients with ischemic stroke, the number of neutrophils circulating in the peripheral blood circulation will increase within a few hours after a stroke. This increase is related to stroke severity and infarct volume, which can increase the severity and cause a poor outcome in patients with acute ischemic stroke.¹⁹ The results of previous studies



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

mentioned that NLR has an 86% accuracy rate, 85% sensitivity and 86% specificity in predicting outcomes in acute ischemic stroke patients.²²

In this study, there is a significant correlation between hsCRP and severity of acute ischemic stroke ($r = 0.896$; $p = 0.001$). Relevant results also obtained by Singh et al (2019), in 60 patients with acute ischemic stroke are found an increased CRP level in the first day of admission, this increase has a correlation to severity level in acute ischemic stroke patients with NIHSS score is 15-24 and > 25 ($r = 0.4645$, $p = < 0.05$).²⁰ Similar results also found in the study of Yu et al (2016) which stated that increased hsCRP levels are correlated to the severity in acute ischemic stroke patients.²¹ In this study, there is a significant correlation between hsCRP and acute ischemic stroke outcome ($r = 0.624$, $p = 0.001$). It is relevant to study that conducted by Putri et al (2017) which stated that there is a significant correlation between hsCRP levels and mRS scores for ischemic stroke outcomes ($r = 0.585$; $p = 0.001$). Specifically, this shows that increased hsCRP levels can predict poor outcomes in acute ischemic stroke patients with 88% accuracy, 95% sensitivity, and 81% specificity.²² In a recent study that conducted by Kumar et al (2020) in 54 patients with acute ischemic stroke, results showed that hsCRP has a significant correlation to mRS score for ischemic stroke outcomes after 90 days ($p = 0.0003$).²⁴

Increased CRP levels in acute stroke significantly indicate an inflammatory response to acute stroke. Increased CRP levels have been linked to the occurrence of extensive infarction and bleeding, severe neurological deficits, causing increased severity level in patients. The process of acute inflammation with tissue damage can stimulate the production of CRP. CRP itself is an acute glycoprotein produced by the liver. hsCRP has 88% accuracy rate, 95% sensitivity, and 81% specificity in predicting outcomes in acute ischemic stroke patients.²² Increased CRP levels have a function to bind phosphocholine on the surface of dead cells as an opsonin and activate the complement.²³

Increased CRP levels can occur between 12-24 hours after onset of the first attack and can reach peak levels after 36-72 hours. Some studies said that CRP contributes to secondary brain damage after ischemic stroke through the process of complement activations. CRP will have an excessive binding to ligands that are exposed to death and damaged cells so that it can stimulate the activation of the complement substance by releasing chemotactic factors and cell opsonization in surrounding lesions which leads to an increased inflammatory cell infiltration as a result of damage to surrounding tissue that can cause worsening of patient outcomes.²⁵

This study has several limitations, assessment of hsCRP and RNL levels only performed once on the first day of admission. Assessment of hsCRP and RNL levels was performed in patients with different onset of ischemic stroke. And in this study, there was no reevaluation of outcomes in patients after 90 days post-treatment.

Conclusion

There is a significant correlation between NLR and hsCRP with severity and outcome in acute ischemic stroke patients.

Suggestion

Taking and examining samples of hsCRP serum and NLR more than once to be able to see changes in samples of hsCRP serum level and NLR. NLR and of hsCRP serum in every patient should be measured on the same onset of acute ischemic stroke. In the last one, it is necessary to monitor the outcome for 3 months so it can provide more representative results.

References

- [1] Robbins, S.L., Kumar, V., and Cotran, R.S. 2017. *Pathology*. 9th Ed. Vol 1, EGC. Jakarta.
- [2] Vural, G., Gumusyayla, S., and Akdeniz, G. 2018. Neutrophil Lymphocyte Ratio and its Relationship with Functional Recovery in Stroke Patients. *Medeniyet Medical Journal*. 33(4): 320-25.
- [3] Yu, S., Arima, H., Bertmar, C. Clarke, S., Herkes, G., and Krause, M. 2018. Neutrophil to Lymphocyte Ratio and Early Clinical Outcomes in Patients with Acute Ischemic Stroke. *Journal of the Neurological Sciences*. 387: 115-18.
- [4] Zhang, J., Ren, Q., Song, Y., He, M., Zeng, Y., Liu, Z., et al. 2017. Prognostic Role of Neutrophil-Lymphocyte Ratio in Patient with Acute Ischemic Stroke. *Medicine*. 96(45): 1-5.
- [5] Xue, J., Huang, W., Chen, X., Li, Q., Cai, Z., Yu, T., et al. 2017. Neutrophil- to-Lymphocyte Ratio Is a Prognostic Marker in Acute Ischemic Stroke. *Journal of Stroke and Cerebrovascular Disease*. 26(3):



INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

650- 57.

- [6] Huang, Y., Jing, J., Zhao, X., Wang, C., Wang, Y., Liu, G., et al. 2012. High Sensitivity C-Reactive is a Strong Risk Factor for Death After Acute Ischemic Stroke among Chinese. *CNS Neurosciences and Therapeutics*. 18(3): 261-6.
- [7] Pandev, S., Kawre, K.K. and Dwifedi, P. 2018. Prognostic Significance of hsCRP in Acute ischemic stroke patients. *International Journal of Advances in Medicine*. 5(3): 578-82.
- [8] Roubary, S.A., Saadat, F., Forganpharast, K., and Sohrabnejad, R. 2011. Serum C- Reactive Protein Level as a Biomarker for differentiation of Ischemic Stroke from hemorrhagic Stroke. *Acta Medica Iranica*; 49(3): 149-52.
- [9] Chaudhuri, J.R., Mridula, K.M., Umamahesh, M., Swathi, A., Balaraju, B. and Bandaru, V.C.S. 2013. High Sensitivity C-Reactive Protein levels in Acute Ischemic Stroke and Subtypes: A Study from tertiary Care Center. *Iranian Journal of Neurology*. 12(3): 92-7.
- [10] Rambe, A.S., Fithrie, A., Nasution, I. and Tonam. 2012. *Profile of Stroke Patients at 25 Hospitals in North Sumatra 2012, Hospital Based Survey*. *Neurona*: 30(2).
- [11] Boehme, A., Esenwa, C. and Elkind, M. 2017. Stroke Risk Factors, Genetics, and Prevention. *Circulation Research*. 120(3): 472-95.
- [12] Reilly, M. R. and McCullough, L. D. 2018. Age and Sex Are Critical Factors in Ischemic Stroke Pathology. *Endocrinology*. 159(8):3120–3131
- [13] Bushnell, C., McCullough, L.D., Awad, I.A., Chireau, M.V., Fedder, W.N., Furie, K.L., et al. 2014. Guidelines for The Prevention of Stroke in a Women. Statement for Healthcare Professionals From The American Heart Association / American Stroke Association. *Stroke Journal of The American Heart Association*. 45(5) :1-45.
- [14] Basic Health Research. 2013. Indonesian Ministry of Health's Health Research and Development Agency
- [15] Swinstonska, M., Slomka, A., Korbal, P., Slomka, N.P., Sienkiewicz, W. Sokal, P., et al. 2019. Association of Neutrophil-to-Lymphocyte Ratio and Lymphocyte-to-Monocyte Ratio with Treatment Modalities of Acute Ischaemic Stroke: A Pilot Study. *Medicina*. (55)342: 1-12
- [16] Hunaifi, I and Cahyawati, T.D. 2019. *Correlation Between Lymphocyte Neutrophil Ratio and the Cerebral Infarction volume in Patients with Acute Ischemic Stroke*. *Neurona*. (36)4:289-92
- [17] Yu, S., Arima, H., Bertmar, C. Clarke, S., Herkes, G., and Krause, M. 2018. Neutrophil to Lymphocyte Ratio and Early Clinical Outcomes in Patients with Acute Ischemic Stroke. *Journal of the Neurological Sciences*. 387: 115-18
- [18] Jin, R., Yang, G. and Li, G. 2010. Inflammatory Mechanism in Ischemic Stroke: Role of Inflammatory Cell. *Journal of Leukocyte Biology*. 87(5): 779-89.
- [19] Jickling, G.C., Liu, D., Andir, B.P., Stamova, B., Zhan, X., and Sharp, F.R. 2015. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *Journal of Cerebral Blood Flow and Metabolism*. 35(6): 888-901.
- [20] Singh, D.B., Kalra, D.S., Saxena, D.G.N., Singh, D.P., and Vij, D.A. 2019. To The Correlation of hsCRP and ESR with Severity of Acute Ischemic Stroke: A Case Control Study. *Journal of Medical Science and Clinical Research*; 7(6): 841-47.
- [21] Yu, H., Huang, Y., Chen, X., Nie, W., Wang, Y., Reed, G.L., et al. 2016. High Sensitivity C-reactive Protein in stroke patients The Importance in Consideration of Influence of Multiple Factors in The Predictability for disease severity and death. *Journal of Clinical Neuroscience*. 36: 12-19.
- [22] Putri, T.K., Ganie, R.A. and Rambe, A.S. 2017. Neutrophil Lymphocyte Ratio and High Sensitivity C-Reactive Protein as Ischemic Stroke Outcome Ratio. *Journal of Indonesian of Clinical Pathology and Clinical Laboratory*. 23(3): 240-45.
- [23] Gondowadjaja, Y. 2014. *High CRP serum levels in patients with acute ischemic stroke as a predictor of poor outcomes during the treatment*. Udayana University. Denpasar.
- [24] Kumar, S.R. and Vidya, T.A. 2020. High sensitivity C-Reactive Protein Level in Cerebrovascular Accident. *International Journal of Advances in Medicine*. 7(4): 666-72.
- [25] Di Napoli, M., Elkind, M. S., Godoy, D.A., Singh, P., Papa, F., and Wagner, A.P. 2011. Role of C-Reactive Protein in Cerebrovascular Disease. *Expert Review Cardiovascular Therapy*. 9(12):1565-84.