

Correlation Between Number of Leucocyte and CRP Levels with Infarct Volume of Acute Ischaemic Stroke

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ABSTRACT

Aim: To determine the correlation between the inflammatory response with the size of the infarct of the first acute ischemic stroke.

Study design: Cross sectional study.

Methods: Blood samples were taken in the ward. CT scan was performed when the patient entered emergency room. Infarct volume measured by manual tracing perimeter infarct. The correlation analysis used various statistical tests and multivariate tests with logistic regression.

Results: Obtained subject as many as 43 sample. The mean of infarct volume was $3.04 \pm 6.95 \text{ cm}^3$, leukocytes was $1,0120.5 \pm 3,444.9 / \text{mm}^3$ and hsCRP was $2.49 \pm 5.3 \text{ mg / dL}$. There is a positive correlation between leukocyte count and infarct volume but the correlation is weak, whereas CRP levels with infarct volume showed a positive and significant correlation. Multivariate test for risk factor resulted in correlation between CRP and BMI levels with infarct volume but only CRP showed significant correlation ($p = 0.022$).

Conclusions: There is a weak positive correlation between the number of leukocytes and the volume of acute stroke infarction. There is a significant correlation between CRP levels and the volume of acute stroke infarction.

Keywords: number of leukocytes, CRP levels, infarct volume

INTRODUCTION

Stroke is the third leading cause of death after cardiovascular disease.¹ Data in Indonesia shows a trend of increased stroke cases in terms of death, incidence and disability.² In 2013, the prevalence of stroke increases with age, the highest age is ≥ 75 years and there is similar prevalence between men and women.³

The majority of cerebral stroke is ischemic stroke, approximately 85%, caused by occlusion of main cerebral artery by thrombus or embolism. There is an increased difference between men and women in relation to stroke, not only in terms of stroke risk, but also in terms of etiology, symptoms, and outcomes.⁴

Common stroke risk factors include hypertension, diabetes mellitus, smoking, and heart disease. But it does not fully cover all the risk factors for stroke, and stroke patients, especially young people, often do not have these factors.⁵

Inflammatory reactions are closely related to infarct stroke. Inflammatory reactions or responses to infarct strokes begin to be widely studied, to improve patients' outcomes, but still give different results.⁶ Inflammatory responses may aggravate brain parenchymal damage.⁷

Neuronal death will lead to activation of microglia. Activated microglia will produce proinflammatory molecules, leading to the recruitment and infiltration of peripheral leukocyte cells into the brain parenchyma.⁸ On the other hand, the damage to the blood-brain barrier from an ischemic stroke will result in the brain's parenchymal exposure by peripheral circulation and allow invasion of peripheral immune cells. They may be in contact with dead neurons in the brain.⁹ During cerebral infarction there is an increase in hematopoiesis activity which will increase the

number of inflammatory cells in the peripheral circulation in response to the process of recruitment of inflammatory cells into the brain.¹

The systemic concentration of a number of inflammatory markers has been associated with stroke events.¹⁰ Moderately elevated CRP may reflect chronic low-grade inflammation and some studies have concluded that there is a significant increased risk of stroke in patients with increased CRP concentrations.¹¹ A recent study has questioned the usefulness of CRP as an independent predictor of the risk itself, suggesting that CRP may be a surrogate marker for other cerebrovascular risk factors.¹² IL-6 is a major inflammatory cytokine involved as a predictor of stroke risk, the leukocyte activity being an overview of inflammation and an increase in the total number of leukocytes and neutrophils is a predictor of the occurrence of a first stroke or recurrent stroke.^{13,14}

Some trials support that inflammatory responses play an important role in the outcome of infarct stroke patients, and are often associated with more severe brain damage. In particular, early leukocytosis and neutrophilia were associated with the volume of infarction.¹⁵ Other studies suggest an increase in the number of leukocytes and peripheral neutrophils associated with higher recurrent stroke infarction.¹⁶ The infarct volume in parenchyma causes impaired stroke infarction, which is reflected in the disability as a result from the death of neurons.¹⁷

The purpose of this study is to determine the correlation between the number of leukocytes and CRP levels with the volume of acute ischemic stroke infarction.

METHODS

This study was an observational study with a cross-sectional approach for acute ischemic stroke patients, which is conducted in the ward of RSUP dr. Kariadi Semarang and RSUD dr. Soeselo, Slawi, Central Java, on a consecutive sampling. Start from January to May 2017. This study has

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received approval from the Medical Research Ethics Commission FK UNDP / RSUP dr. Kariadi Semarang.

The inclusion criteria of the study subjects were all patients with acute ischemic stroke based on anamnesis, physical examination and non-contrast head CT scans, first stroke and age ≥ 45 years. Exclusion criteria were patients with a history of head trauma, cerebral infections, brain tumors and brain intoxication, the location of more than one infarction or lacuner infarction and infarction in the brain stem.

Questionnaires were performed to obtain data of address, age, gender, education level, occupation, and disease history. After the diagnosis of acute stroke is established, researcher asks for patient's willingness to be included in voluntary research. Blood laboratory tests were performed to look up data on leukocyte count, CRP levels, blood sugar and triglyceride levels. Smoking is obtained from anamnesis while BMI is obtained by measuring height and weight of the body. Data on hypertension were obtained from anamnesa about a history of disease and measurement of blood pressure with sphygmomanometer. Researcher measured the infarct volume by using a Siemens scanning engine with perimeter manual tracing of infarct techniques.

Univariate analysis is performed to descript all research data. Bivariate analysis to test the correlation of leukocyte count, CRP level or confounding factor with acute ischemic stroke infarct volume using Chi-square test with 95% confidence level, Fisher's Exact test and unpaired t test. Multivariate analysis was performed by logistic regression. Presentation and analysis were performed with SPSS for Windows version 22. Statistical values were considered significant if $p < 0.05$.

RESULTS

Obtained 74 subjects of first acute infarction stroke who treated in hospitals dr. Soeselo, Slawi and dr. Kariadi, Semarang. Three subjects were unwilling to follow the study and 28 subjects met the exclusion criteria. A total of 43 subjects who met the inclusion and exclusion criteria followed the study. There was no drop out subject in this study.

In this study, there is balances distribution of subjects of sex, is found in 22 female subjects, 51.2%, and 21 male ones, 48.8%. The average age is 60.49 ± 9.41 years, with the highest age being 86 years while the lowest age is 45 years. The highest number of educational criteria was the group graduated from SMA as many as 17 people, 39.5%. From the marital status there are 1unmarried subjects, 2.3% and 42 of the subjects were married, 97.7%. The most job of subjects 14 were as housewives, 32.6%. Forty subjects had nutritional status (BMI) < 24.9 Kg / cm² (Normoweight), 93%, and 3 had BMI > 24.9 Kg / cm² (overweight / obesity), 7% (Table 1)

As many as 37 subjects suffering from hypertension, 86%, diabetes mellitus 12, 27.9%, smokers were in 12, 27.9%. A total of 21 subjects, 48.8%, suffered from dyslipidemia. EKG examination result from all subjects was found 36 subjects, 83%, had left ventricular hypertrophy and 7 subjects had normosinus rhythm EKG, 16.7%. (Table.1)

From total subjects, the mean infarct volume was 3.04 ± 6.95 cm³. Minimum volume was 0.1 cm³, maximum one was 35 cm³ and the median was 0.8 cm³. There is a large difference in volume value between the maximum volume and the minimum volume with most distributed on small volumes. There were 34 subjects who had a volume below the mean and 9 subjects had a volume above the mean (Table 1).

Table 1. Distribution of samples characteristics (n=43)

Variable	Frequency (%)
Gender	
Male	21 (48.8%)
Female	22 (51.2%)
Age	60 (45-86)(Mean)
BMI	
Obese	3(7%)
Non obese	40(93%)
Marital state	
Married	42 (97.7%)
Not married	1(2.3%)
Education	
Elementary school	8 (18.6%)
Junior high school	14 (32.6%)
Senior high school	17 (39.5%)
University	4 (9.3%)
Job status	
Housewife	14 (32.6)
Farmers	6 (14.0%)
Private employes	9 (20.9 %)
Government employes	9 (20.9%)
Soldier/police	2 (4.7%)
Others	3 (7 %)
ECG	
LVH	36(83.7%)
NSR	7(16.3%)
Hypertension	
Yes	37(86%)
No	6(14%)
Diabetes mellitus	
Yes	12(27.9%)
No	31(72.1%)
Dyslipidemia	
Yes	21(48.8%)
No	22(51.2%)
Smoking	
Yes	12(27.9%)
No	31(72.1%)
Obesity	
Yes	3(7%)
No	40(93%)
hsCRP (mg/dL) level	2.45(0.01-29.12)
Leukocytes (mm³) count	10.120(3.650-23.700)
hsCRP category	
Normal	15(34.9%)
High	28(65.1%)
Leukocytes	
Normal	27(62.8%)
High	16(37.2%)
Infarct volume (cm³)	3.04(0.1-35.0)

The average of leukocytes number from all subjects was $1,0120.5 \pm 3,444.9$ / mm³. The lowest leukocyte count was 3,650 / mm³ and the highest leukocyte count was 23,700 / mm³, 16 subjects, 37%, had high category of

leukocytes and 27 subjects had normal leukocytes, 62.8%.(Table.1)

The mean serum CRP level was 2.49±5.3 mg / dL. The lowest value was 0.01 mg / dL, the highest one was 29.12 mg/dL with mean of 2.49 mg / dL. Nine subjects, 20.9%, had hsCRP levels above mean and 34 subjects, 79.1%, lower than mean, 28 subjects, 65.1%, had high hsCRP and 15 subjects, 34.9%, had normal hsCRP levels.(Table.1)

Researcher obtained data with abnormal distribution on leukocyte count, hsCRP and infarct volume. Data transformation has been performed to obtain a normal distribution, but from various transformations it does not show normality assumption. For statistical calculations, these nine extreme data are not taken into account as they will affect the statistical test results.

Researcher use Bivariate analysis to assess the relation of leukocyte count to infarct volume by using pearson correlation test, and there was a positive correlation between leukocyte count and infarct volume ($r = 0.015$) but the Correlation was not significant ($p = 0.496$) (Fig.1). Furthermore, we conducted a comparative test by categorizing the volume of infarcts into large and small volumes based on the cut of point. Obtained a cut of point infarct volume for leukocyte count and hsCRP levels was 0.76 cm³. Using the fisher comparison test, there was no significant correlation between leukocyte count and infarct volume ($p = 0.715$).

Bivariate analysis for CRP levels and infarct volume using Pearson correlation test, showed a positive correlation between hsCRP levels to infarct volume, $r=0.217$, and the correlation was significant, $p = 0.005$,(Fig.1). Researchers then performed a chi square comparative test with a cut of point for infarction volume of 0.76 cm³ and found a significant association between hsCRP levels and infarct volume, $p = 0.042$.

Table 2. Correlations between confounding factors with infarct volumes

Data characteristics	Infarct volume		P Value
	Large	Small	
Age	18(52.9%)	16(47.1%)	0.113*
BMI			
Obes	3(16.7%)	0	0.230**
Non Obes	15(83.3%)	16(100%)	
Hypertension			
Yes	16(88.9%)	13(81.3%)	0.648**
No	2(11.1%)	5(18.8%)	
DM			
Yes	4(22.2%)	4(25%)	1.0**
No	14(77.8%)	12(75%)	
Dyslipidemia			
Yes	10(55.6%)	5(31.3%)	0.154***
No	8(44.4%)	11(68.8%)	
Smoker			
Yes	3(16.7%)	3(18.8%)	1.0**
No	15(83.3%)	13(81.3%)	

Informations:

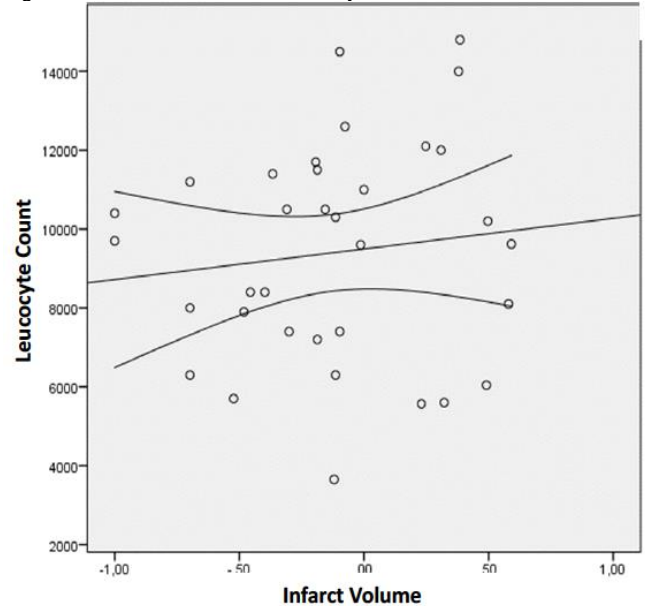
*= Independent t test

**= Fishers comparisons test

***= Chi square comparisons test

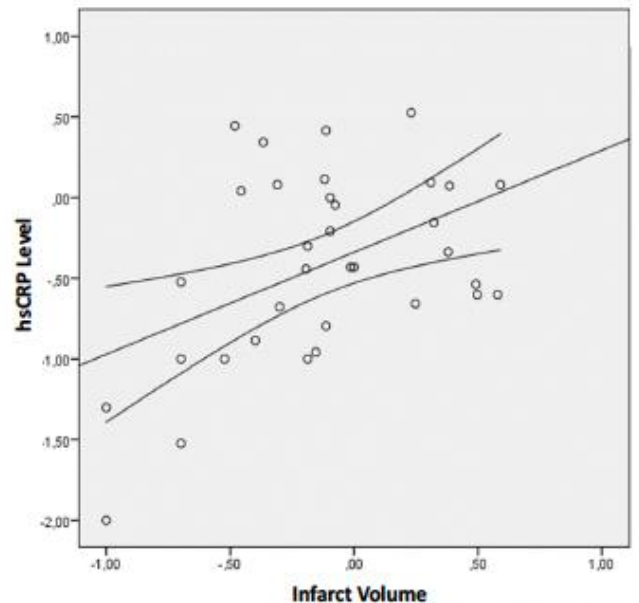
There was no significant correlation between age factor withsize of infarct volume, $p = 0.113$. Similarly, BMI with infarct volume, $p = 0.230$, hypertension with infarct volume, $p = 0.648$, diabetes mellitus with infarct volume, $p = 1.0$, dyslipidemia with infarct volume, $p = 0.154$, and smoking with infarct volume, $p = 1.0$ (Table 2)

Fig. A. Correlations between Leucocyte Count with infarct volumes



Perason's $r=0.015$ $p=0.496$

Fig. B. Correlations between hsCRP Level with infarct volumes



Perason's $r=0.217$ $p=0.005$

In multivariate analysis it was found that BMI and hsCRP levels were associated with large infarct volumes, but BMI was not significant, $p = 0.99$, whereas hsCRP levels had significant Correlation, $p = 0.02$, with OR 6.6. (Table.3)

Table 3. Factors affected infarct volume (Logistic regression test)

Risk factors	Bivariate analysis						Multivariate analysis			
	Infarct volume		P	OR	IK 95%		p	Ex(B)	IK 95%	
	Large	Small			Min	Max			Min	Max
BMI										
Obese	3(16.7%)	0	0.230*	2.07	1.44	2.97	0.999	2x10 ⁹	0.00	-
Non obese	15(83.3%)	16(100%)								
Dislipidemia										
Yes	10(55.5)	5(31.3%)	0.154**	2.75	0.67	11.24	0.543	1.65	0.33	8.28
No	8(44.4%)	11(68.7%)								
hsCRPcategory										
High	13(72.2%)	6(37.5%)	0.042**	4.33	1.02	18.38	0.022	6.67	1.32	33.69
Normal	5(27.8%)	10(62.5%)								

*Fishers comparisons test

**Chi square comparisons test

DISCUSSION

This study was conducted on 43 samples that met inclusion and exclusion criteria. From the gender characteristics, there was a balanced percentage between male and female, 48.8% for males and 51.2% for females. This is in accordance with the Riskesdas report in 2013 where the prevalence of stroke for male gender was 7.1% and women 6.8%. In this study, more than 50% of the samples were aged over 60 years, which was in accordance with Riskesdas report that stroke prevalence increased with age increase.³

Most of the educated sample completed high school by 39.5% and the smallest is 9.3% graduated from university. This is not in accordance with the Riskesdas report in 2013, where the greatest percentage of strokes obtained in primary school education then decreased according to education and then rose again in college education finished. This difference may be due to differences in sample demographics between researchers with Riskesdas in which Riskesdas has broader coverage, whereas researchers only conduct research in two districts³

Risk factors studied were hypertension and EKG respectively 86% and 83.7%, diabetes mellitus 27%, dyslipidemia 48%, and smoking by 27%. In accordance with the Perdossi stroke guideline (2011) stating that the biggest cause of stroke is hypertension, which is about 70% - 94%.³

The leukocyte count was increased at 37.2% of the sample and within normal limits in 62.8% of the sample. HsCRP levels increased at 65.1% of samples and normalized at 34.9% of the sample. Leucocytes and especially CRP are both inflammatory markers where in the study of Guo Y et al in 2013 and Woodruff et al in 2011 agreed to suggest that there was an increase in inflammation in stroke patients.^{18,19} In this study the number of samples with high leukocytes was actually less than the sample with normal leukocytes may be due to a lack of sample size. The acquisition of extreme large values in leukocyte count and hsCRP levels in this study is possible because of the presence of comorbid in the sample, namely lung infection at the time of blood sampling. The infections that are being experienced by the sample will increase the value of the inflammatory marker.^{20,21}

Researcher measure the cerebral infarct volume with CT scan by manual tracing of the perimeter cerebral infarction method. Currently, this method is the best

method for measuring infarct volume.²² Obtained infarct volumes with a mean of $3.04 \pm 6.95 \text{ cm}^3$.³ But we got 4 samples with extreme values on the normality test of the data. The volume obtained is too large compared to the mean. This may be due to comorbid factors but also because of the measurement time distance from the onset of stroke. The four samples were examined for ct scan approaching 72 hours and after 72 hours from onset while other samples performed ct scan within a few hours from onset. Thomas Brott et al in 1989 said that a ct scan for an infarction stroke with an onset of less than 48 hours, the sensitivity was only 40% and the examination within 7 to 10 days after the onset of sensitivity doubled to 77% and would stay up to 3 months after onset.²³

In this study, researcher was founded a positive correlation between increased number of leukocytes with increased infarct volume in Pearson correlation test, but the correlation is weak. In the Fisher test between the leukocyte category and the infarct volume there was no significant correlation between the leukocyte count and the size of the infarct volume. A.J. Grau et al. 2004 stated that leukocytes are independent predictors of stroke,¹³ whereas Zoppo et al in 2014 concluded that leukocytes are responsible for the development of ischemia of brain tissue into infarction and high leukocytes on day 3 of onset associated with poor outcomes of infarct stroke patients.²⁴ The results of this study are less suitable with studies before because leukocyte was not taken on the third day but the first day of hospitalization, and the measurements of infarct volume were not performed at the same time range from onset of stroke.

In this study, there was a significant correlation between hsCRP levels and infarct volume. In the Pearson's correlation test obtained positive correlation between hsCRP levels with infarct volume so that the higher hsCRP value result higher infarct volume, but the correlation of both is weak. Using chi square test, there was a significant correlation between hsCRP and infarct volume, $p = 0.042$. In multivariate analysis, the factors influencing the size of infarct volume were nutritional status (BMI) and hsCRP levels. This is in accordance with some studies that have been done before. F. Epstein in 1999 stated that CRP mediates complement factors that will aggravate inflammatory responses throughout the body including the brain. CRP contributes to the development of atherosclerosis, improves procoagulant status and decreases fibrinolysis.²¹

Limitations of this study are that comorbid infections are not an exclusion criteria, blood sampling is

not in the same time range, time range of CT scan implementation of onset is not the same, did not measure liver function and did not take into account nitric acid.

CONCLUSIONS

There is a weak positive correlation between the number of leukocytes and the infarct volume of acute stroke infarction. There is a significant correlation between CRP levels and the volume of acute infarct stroke. Acute ischemic stroke patients with high CRP levels have a risk of having a large infarct volume of 6.67-fold compared to patients with acute ischemic stroke with normal CRP levels.

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