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## CHARACTERISTIC OF RED BLOOD CELL PROFILE AS A PREDICTOR OF 30-DAY CLINICAL OUTCOME IN ISCHEMIC STROKE PATIENTS

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#### ABSTRACT

Ischemic stroke is the most common type of stroke in Indonesia. Measuring inflammatory biomarkers (IL-6) is a way to predict clinical outcomes in ischemic stroke patients. However, the laboratory test is very experience, so it requires another test that can represent the inflammation, for instance, by measuring Red Cell distribution Width (RDW) and Mean Corpuscular Volume (MCV) value when hospitalized. Hence, this study aims to measure the RDW and MCV value of ischemic stroke patients at admission with a 30-day clinical outcome (disability). This study is a retrospective cohort that used secondary data from medical records of ischemic stroke patients in Bethesda Hospital Yogyakarta and was conducted on 105 subjects with consecutive sampling. They are divided into two groups by their clinical outcome (disability) in 30-day after onset, that be measured by Modified Rankin Scale(mRS), (1) the independent group who had mRS score of 0- 2, (2) the dependent group who had mRS score of 3-6. After that, these two groups are analyzed by an independent t-test. High RDW and low MCV value at admission increased the 30-day disability risk. There is a 17 nificant difference in mean RDW value between independent and dependent groups (p = 0.008), but there is no difference in mean MCV value between independent and dependent groups (p = 0.277). 30-day clinical outcome in ischemic stroke patients can be predicted by their red blood cell profile characteristic at admission.

**Keywords**: Disability; Ischemic Stroke; MCV; Predictor of 30-day Clinical Outcome; RDW

#### INTRODUCTION

A non-communicable disease most commonly found in Indonesia is stroke. West Kalimantan (14.7%) and Yogyakarta (14.6%) are the top two provinces with the highest prevalence. 15 out of 1000 Indon sian people suffer from stroke. I Ischemic stroke is a deficit of neurologic function due to loss of blood circulation to the brain, and this type of stroke is the most common in Indonesia. 2

Loss of blood circulation to the brain causes ischemic and leads to infarct. The infarct size correlates with inflammation and can be known by measuring the blood's proinflammatory markers (IL-6). A cohort study conducted by Aref et al. (2020) reported that 21 of 26 ischemic stroke patients who had high IL-6 in the blood (> 7.75 pg/mL)

increased the risk of poor clinical outcome.<sup>3</sup> High IL-6 can be found until 90-day after onset. <sup>4-6</sup>

Inflammation will affect the character of the red blood cell profize where patients with high IL-6 will have an RDW (Red Blood Cell Distribution Width) value of more than 15%, and the N25V value is still under debate. Hatamian *et al.* (2014) conducted a cross-sectional study and reported that increasing 1 21 to f MCV value decreased the risk of poor clinical outcomes in ischemic stroke patients. Meanwhile, a cohort study that was conducted by Wu *et al.* (2018) stated that high MCV value had poor clinical of come.<sup>7-9</sup>

One example of a clinical outcome in ischanic stroke patients is a disability that can be measured by mRS (Modified Rankin Scale) score and its scale from 0 (no

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symptoms) to 6 (death). This scoring system indicates their disability when their score is measured and strongly correlates with the pathology of stroke (infarct's size). <sup>10-12</sup> Therefore, this study measured RDW and MCV values at admission and 30-day clinical outcome (disability) in ischemic stroke patients.

### MATERIAL AND METHODS

This study was a retrospective cohort and was congested on 105 ischemic stroke patients at Bethesda Hospital Yogyakarta from 2019 until 2020, using their medical records from the stroke registry.

The Independent variable in this study was characteristic of red blood cell profile (RDW and MCV value) at admission, and the dependent variable was 30-day disability (mRS score at 30-day after onset).

The inclusion criteria in this study were as follows: (1) patients who had been diagnosed for the first time with mild or moderate acute ischemic stroke, (2) patients who do laboratory tests for the characteristic of red blood cell profile (RDW and MCV value) at admission (3) patients who had been following up until 30-day after onset, while the exclusion criteria in this study were as follows: (1) patients known to have iron deficiency and megaloblastic anemia (2) patients known to have another chronic inflammatory disease (3) patients had an acute complication (pneumonia) (4) pregnant women (5) loss to follow up (6) patients had passed away before 30-day after onset (7) patients who insisted to be discharged or refer to another hospital (8) data was not completed.

The data analysis was performed using the Independent t-test to compare the mean difference between RDW and MCV value between independent (mRS score 0-2) and dependent groups (mRS score 3-6).

This study has complied with all regulations based on The Council for International Organizations of Medical Sciences (CIOMS) 2016 and has received ethical approval from Bethesda Hospital Yogyakarta's Health Research Ethics

Commission with ethical approval number No. 56/KEP-RSB/XI/21.

#### RESULT

One hundred five subjects were divided into two groups by their mRS score 30-day after onset, (1) independent groups who had mRS scores of 0-2 (92 subjects), (2) dependent groups who had mRS scores of 3-6 (13 subjects).

#### **Basic Subjects' Clinical Characteristics**

The basic Subjects' Clinical Characteristics are shown in Table 1. At admission, the subjects were classified into two groups by their RDW and MCV values.

Male subjects dominated in this study, and the mean age of each group was different (62.061 vs. 58.428). The most common comorbidities in these two groups were hypertension and diabetes mellitus. The infarct's locations were variated from each group, (1) supratentorial (83 subjects), (2) infratentorial (7 subjects), and the rest of the subjects. Their infarct locations were not visualized yet with a Non-Contrast MSCT Scan (15 subjects).

Table 2 shows the results of the data analysis. The mean RDW value at admission in independent groups was lower than in dependent groups (12.8609% vs. 13.9615%), indicating that a high RDW value increased the risk of poor clinical outcomes. The mean MCV value at admission in independent groups was higher than in dependent groups (84.8554 fL vs. 82.4615 fL), which indicated low RDW value increased the risk of poor clinical outcomes.

Table 1. Basic Subjects' Clinical Characteristics

RDW and MCV value at	1. Basic Subjects' Clinical RDW ≤ 14.6%,	RDW > 14.6%,	P value
admission	$MCV \le 14.0 \%$ , $MCV \le 98 \text{ fL}$	$MCV \le 98 \text{ fL}$	1 value
	Me v = yo IL	Me v _ you	
N	98	7	
Gender, n (%)	76	'	0.873
	-0 (50 A)		
Male	59 (60.2)	4 (57.14)	
Female	39 (39.8) 62.061±10.776	3 (42.86) 58.428±9.998	0.389
Age, years	62.061±10.776	38.428±9.998	0.389
Comorbid, $n$ (%)			
Hypertension	50 (51.02)	4 (57.14)	0.754
Diabetes Mellitus	34 (34.69)	2 (28.57)	0.742
Cardiovascular disease	17 (17.35)	1 (14.28)	0.836
Gastrointestinal disease	4 (4.08)	0 (0)	0.586
B 11 11 1	1 (1.02)	0 (0)	0.700
Dyslipidemia	1 (1.02)	0 (0)	0.788
Hypokalemia Thrombocytosis	1 (1.02) 1 (1.02)	0 (0) 0 (0)	0.788 0.788
Thiombocytosis	1 (1.02)	0 (0)	0.766
COPD	1 (1.02)	0 (0)	0.788
NIHSS, $n$ (%)			0.523
Mild impairment	40 (40.8)	2 (28.57)	
Moderately severe impairment	58 (59.2)	5 (71.43)	
Location of infarct, $n$ (%)			
Supratentorial	76 (77.55)	7 (100)	0.159
Infratentorial	7 (7.14)	0 (0)	0.464
mRS score in 30-day after onset $n(\%)$			0.011
0-2 (independent groups)	88 (89.8)	4 (57.14)	
3-6 (dependent groups)	10 (10.2)	3 (32.86)	
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Abbreviations: RDW: Red Cell Distribution Width; MCV: Mean Corpuscular Volume; NIHSS: NIH Stroke Scale; mRS: Modified Rankin Scale; COPD: Chronic Pulmonary Disease.

Table 2. Independent t-Test Results

Table 2. mucpendent t- Test Results						19
Independent Groups			Dependent Groups			P value
n	Mean	SD	n	Mean	SD	_
92	12.8609	0.98772	13	13 9615	2.03205	0.008
	12.000)	0.50772	15	15,5015	2.00200	0.000
92	84.8554	5.18201	13	82.4615	7.33877	0.277
	n 92 92	n Mean 92 12.8609	n Mean SD  92 12.8609 0.98772	n Mean SD n 92 12.8609 0.98772 13	n         Mean         SD         n         Mean           92         12.8609         0.98772         13         13.9615	n         Mean         SD         n         Mean         SD           92         12.8609         0.98772         13         13.9615         2.03205

Abbreviations: RDW: Red Cell Distribution Width; MCV: Mean Corpuscular Volume; SD: Standard of Deviation.

#### **DISCUSSION**

RDW (Red Cell Distribution Width) is a parameter that shows the size variation of erythrocytes. At the same time, MCV (Mean Corpuscular Volume) is a parameter that shows the mean of erythrocytes' size and volume. 13-14 These two values divers due to inflammation in the body. Inflammation occurred in ischemic stroke and caused erythropoiesis impairment. This study found that the RDW value of dependent groups was higher than independent groups, or a high RDW value incressed the risk of 30-day disability with a statistically significant pvalue (p-2005). These results were similar to another study by Sarhan et al. (2019), in which the reported mean of RDW value in ischemic stroke patients was higher than in the control groups (15.4% vs. 13.66%). 15 Yogitha and Kumar (2019), Turcato et al. (2017), and Kim et al. (2021) also had the same results. 16-

Meanwhile, the MCV value of dependent groups was lower than independent groups that indicated low RDW value increased the sk of 30-day disability without any statistically significant p-value (p>0.05) because of too many variations in subjects (standard of deviations >2) and the subjects were only mild and moderate stroke, so the distribution of data was uneven. Low MCV with poor clinical outcome in ischemic stroke was also found in Hatamian *et al.* (2014) 's study, but it contradicted the cohort study conducted by Wu *et al.* (2018).<sup>8-9,19</sup>

Inflammation is characterized by an increase in pro-inflammatory markers, namely IL-6, which will cause anisocytosis due to immature erythrocytes released into the circulation and leading to an increase in RDW values which will result in erythrocyte damage, increased erythrocyte fragility, and disruption of erythrocyte maturation. 20-21

Because the increase in the RDW value is caused by inflammation and oxidative stress, therapy car 23 e given to treat both of these. However, it is still difficult to find suitable drugs for inflammation due to the many variations in the immune response and other influencing factors. Drieu et al. (2018)

reviewed the previous research on drugs, including rhIL-1Ra (recombinant IL-1 receptor antagonist), minocycline, enlimomab, natalizumab, Hu23F2G, and UK-279, 276. 22 rhIL-1Ra injected subcutaneously did not significantly reduce the risk of disability (mRS score), while for other drugs, drugs were discontinued during clinical trials or had bad effects. 23

Antioxidants can be given to reduce oxidative stress, one of the antioxidants that have been shown to reduce the extent of infarcts in ischemic stroke is polyphenols. Types of polyphenols include flavonoids, caffeic acid, and curcuminoids. Flavonoid derivatives that can reduce the severity of neurological deficits and the extent of infarcts in ischemic stroke are Baicalein and Epigallocatechin-gallate (EGCG), where EGCG is a polyphenol contained in green tea.<sup>24</sup> Caffeic acid will affect the 5-lipooxygenase enzyme, which will play a role as oxidation.25 catalyst for lipid Tetrahydrocurcumin and Hexahidrocurcumin are part of curcumin which has been shown to reduce the extent of infarcts in the brain.<sup>26</sup>

The decrease in MCV value is caused by increased hepcidin when inflammation occurs. Hepcidin will interfere with erythropoiesis due to the release of iron from the liver and inhibiting iron uptake through enterocytes. <sup>27</sup> Increa od IL-6 will also inhibit the proliferation of BFU-E (Burst-Forming Units-Erythroid) and CFU-E (Colony-Forming Units-Erythroid). Erythroid), which will reduce the MCV value. <sup>28</sup>

A high RDW and low MCV value indicate the presence 26 of hypoxia on endothelial cells, where based on the results of the research of Khongkhatithum et al. (2019), it is known that the group of subjects who have high RDW and low MCV values has high thrombomodulin levels. High thrombomodulin will cause more thrombinthrombomodulin complexes that activate TAFI (Thrombin Activatable Fibrinolysis Inhibitor). where TAFI will inhibit endogenous fibrinolysis and cause thrombosis. Increased TAFI is one of the risk factors for brain infarction.29 This has also

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been proven by a study by Denorme et al. (2016), wherein experimental animal mice were given TAFI inhibitor drugs that could reduce the extent of infarcts by 50%.<sup>30</sup>

The limitations of this study were that the severity of the studied stroke was only mild and moderate, and only 1 form of 30-day clinical outcome appeared, namely disability.

#### CONCLUSION

High RDW are low MCV value at admission increase the risk of disability in ischemic stroke patients. It can be used as one of the considerations to determine the 30-day clinical outcome that appears. In future studies, clinical outcomes can be studied for up to 90 days and are not limited to only mild to moderate strokes. Other forms of clinical outcomes, such as mortality, can be investigated.

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