Correlation between Cycle Threshold (CT) Values with Severity of Clinical Symptoms in COVID-19 Patients

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ABSTRACT

Objective: This study aimed to determine the correlation between Cycle Threshold (CT) value with severity of clinical symptoms in *Corona Virus Disease 2019* (COVID-19) patients at the time of admission.

Design: A survey cross sectional study was conducted in two hospitals: Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar, Indonesia, involved 270 subjects from May to July 2020.

Materials and Methods: Medical records which met the study criteria were included in this study. CT value data was collected and compared among different clinical symptom severity. The correlation was analysed using SPSS version 25. The statistical tests used were Anova and Chi Square test. A multivariate analysis was conducted using multinomial logistic regression.

Results: The mean CT value was significantly lower in severe group and higher in mild group (p < 0.05). We also found a significant correlation between gender, age, comorbidities, and radiological features with the severity of clinical symptom (each p < 0.01) and proceed to the multivariate analysis. The variables which significantly related to severity of clinical symptom were the radiological features of pneumonia (p < 0.001), comorbidities (p < 0.01), and CT value (p < 0.05).

Conclusions: The CT value has significant negative correlation with the severity of clinical symptoms in COVID-19 patients at the time of admission. Adjusted by other variables, CT value still has a significant association with severity of COVID 19 symptom.

KEY WORDS

COVID-19, cycle threshold, severity, viral load

INTRODUCTION

An outbreak of pneumonia cases with clinical signs very similar to viral pneumonia has been globally spread from China to many countries[1,2]. In Indonesia, the series of cases were first reported on March 2, 2020[3]. The cases continued to increase and extent rapidly throughout the country, to date reaching more than 1.6 million confirmed cases[3,4]. The majority of confirmed patients were asymptomatic to moderate with 5.0% of patients were admitted to Intensive Care Unit (ICU)[2].

Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assays is recommended for the diagnosis of *Severe Acute Respiratory Syndrome Coronavirus* 2 (SARS-CoV-2) infection[2]. The diagnosis of COVID-19 is determined based on the CT value[5]. The amount of viral RNA copies is inversely proportional to the CT value[6]. There were numerous studies regarding the relationship between CT value and disease severity[7]. However, only a few reports utilised large samples and most of them were not separated the symptoms into asymp-

tomatic to severe group. There were differences in the methods and results among researchers regarding the association of CT value with the disease severity. Liu *et al*. (2020)[8], Yu *et al*. (2020)[9], and Zheng *et al*. (2020)[10] reported that patients who severely ill had significant higher viral loads (or lower CT value) than mildly ill patients. In contrast to the three previous mentioned studies, Shah *et al*. (2020)[11] reported no difference in CT value of the patients with severe symptoms and those with mild symptoms. This study aimed to determine the correlation between CT value and severity of clinical symptoms in COVID-19 patients from May to July 2020 at Wahidin Sudirohusodo Hospital Makassar and Hasanuddin University Hospital Makassar. Additionally, the other clinical characteristics (gender, age, comorbidities, and radiological features) as well as the CT value were included into multinomial logistic regression model to assess wheter the CT value has significant association adjusted by other variables.

Table 1: Personal and Clinical Characteristics of Patients with Confirmed COVID-19 by Severity of Clinical Symptom at Admission

Variable			Total	Severity of Clinical Symptom				P value
				Asymptomatic	Mild	Moderate	Severe	
Sex	Male	n	123	40	33	29	21	0.005
		%	100.0	32.5	26.8	23.6	17.1	
	Female	n	147	72	43	21	11	
		%	100.0	49.0	29.3	14.3	7.5	
Age	< 60 years	n	219	104	63	37	15	0.000
		%	100.0	47.5	28.8	16.9	6.8	
	≥ 60 years	n	51	8	13	13	17	
		%	100.0	15.7	25.5	25.5	33.3	
Comorbidities	None	n	203	102	59	32	10	0.000
		%	100.0	50.2	29.1	15.8	4.9	
	Yes	n	67	10	17	18	22	
		%	100.0	14.9	25.3	26.9	32.9	
Radiological Findings	Non-Pneumonia	n	144	85	46	10	3	0.000
		%	100.0	59.0	31.9	6.9	2.1	
	Pneumonia	n	126	27	30	40	29	
		%	100.0	21.4	23.8	31.7	23.0	

Table 2: Comparison of Mean CT value According to Severity of Clinical Symptom

Severity of Clinical Symptom	n	Mean	Std. Deviation	p value
Asymptomatic	112	32,1	6,7	
Mild	76	32,9	4,7	0,026
Moderate	50	32,5	4,8	
Severe	32	29,3	5,6	

Table 3: Association between CT value and Severity of Clinical Symptom

CT value Category		Severity of Clinical Symptom				Total
		Asymptomatic	Mild	Moderate	Severe	
<= 29	n	29	12	15	15	71
	%	25.9	15.8	30.0	46.9	26.3
30-37	n	56	55	27	15	153
	%	50.0	72.4	54.0	46.9	56.7
38-40	n	27	9	8	2	46
	%	24.1	11.8	16.0	6.3	17.0
Total	n	112	76	50	32	270
	%	100.0	100.0	100.0	100.0	100.0

Chi Square test (p = 0,003)

MATERIAL AND METHODS

Research Design

This study used a retrospective observational method at Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital, Makassar, Indonesia.

Research Subjects

Research subjects were patients diagnosed with COVID-19 who were admitted to Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital from May to July 2020. Inclusion criteria were the patient's medical records confirmed with COVID-19 (RT-PCR positive), availability of the CT value result and complete medical record data.

Data Collection

The study subjects were taken from medical record data of COVID-19 patients using a total sampling technique. Data regarding personal and clinical characteristics, as well as CT value data were collected. Clinical symptoms were classified into four groups of severity based on Update WHO Recommendation on COVID-19 for Hospital (2020) and Guidelines for Management of COVID-19 (2020): asymptomatic (RT-PCR positive, without any clinical symptoms and signs); mild (with symptoms of uncomplicated upper respiratory tract infection such as cough, fever, sneezing, sore throat, runny nose, nasal congestion, headache, fatigue, anorexia, malaise, or muscle pain, patients may also present with digestive symptoms such as nausea, vomiting, abdominal pain, or diarrhea); moderate (with pneumonia with no signs of hypoxemia); and severe (with pneumonia, plus respiratory rate > 30 breaths/min or SpO2 ≤ 93%). The CT value was categorized into ≤ 29, 30-37, ≥ 38 and compared to the four groups of clinical symptom severity. We also compared the mean CT value at the four groups of clinical symptom severi-

ty. The personal and other clinical information were assessed to obtain their association with the clinical symptom severity. Although the study was conducted in two different hospitals, the cut-off CT value in both hospitals were the same, 40.

Data Analysis

Data analysis was performed using SPSS version 25. The statistical tests used were Anova and Chi Square test. A multivariate analysis was conducted using multinomial logistic regression. The results were considered significant if p-value < 0.05. Several factors (gender, age, comorbidities, and radiological features) were included into multinomial logistic regression model to assess wheter the CT value has significant association adjusted by other variables.

Ethical Clearance

This study protocol was approved by the Health Research Ethics Commission of Hasanuddin University, Medical Faculty, following the ethical recommendations with approval letter number: 775/UN4.6.4.5.31/PP36/2020.

RESULTS

This study included 270 patients confirmed with COVID-19 who

Table 4: Multivariate analysis of Variables on Severity

Variabel	Model Fitting Criteria	Likelihood Ratio Tests		
	-2 Log Likelihood of Reduced Model	Chi-Square	df	p*
Intercept	205,940	0,000	0	.
Gender	211,929	5,989	3	0,112
Age	212,901	6,961	3	0,073
Comorbidities	220,340	14,399	3	0,002
Radiological Findings	248,679	42,738	3	0,000
CT value	218,664	12,723	6	0,048

*Multinomial Logistic Regression

were admitted from May to July 2020. The subject characteristics are shown in Table 1. Most of the subjects were female (54.4%) and age < 60 years (81.1%). Less than half had comorbidities (24.8%) and radiological features of pneumonia (46.7%). Majority of the subjects had CT value 30-37 (56.7%) and a total of 41.5% were asymptomatic. The comparison of mean CT value based on the severity of clinical symptom can be seen in Table 2. Mean CT value was significantly lower in the severe group and higher in the mild group ($p < 0.05$). The correlation between CT value and severity of clinical symptom was shown in Table 3. The mean CT value of severe cases was significantly lower than those of mild cases at the time of admission ($p < 0.01$). Gender, age, comorbidities, and radiological finding also have significant correlation with the severity of clinical symptom and proceed to the multivariate analysis. Based on multinomial logistic regression analysis (Table 4), the variables which significantly related to the severity of clinical symptom were the radiological features of pneumonia ($p < 0.001$), comorbidities ($p < 0.01$), and CT value ($p < 0.05$).

DISCUSSION

There were 270 study subjects which met the study criteria. We observed that more cases were found in female, as observed by Zhang *et al*. (2020)[12] who found 51.6% of their study subjects were female. In contrast, Karyono *et al*. (2020)[13] reported the proportion of male patients were more than female. However, recent research showed there were no significant differences of sex ratio in COVID-19 patients due to the fast spreading of this disease worldwide[14].

In this study, the lowest mean CT value (29.3) found in severe symptom group and the highest (32.9) in the mild symptom group. It showed a significant relationship between CT value and clinical symptom severity in COVID-19 patients at admission ($p < 0.05$). Similar result was also presented in other studies by Liu *et al*. (2020)[8] and Yu *et al* (2020)[9] where the severe cases had significantly lower CT value than mild cases. Zheng *et al*. (2020)[10] also reported that the severe disease had significantly higher viral loads than mild disease. In contrast, Shah *et al*. (2020)[11] observed no difference in CT value between severe and mild cases. This might be due to the vast different number of samples. Viral loads can be detected higher soon after symptom onset and peaked approximately 10 days later[15]. However, there are several factors which may affect the viral load peak, such as treatment received and host clearance[16]. In this study, we also observed the mean CT value in asymptomatic group was lower than in mild group. This is probably because the progression of disease was not observed, therefore it cannot be concluded whether the patient remained asymptomatic or developed symptoms after admission. Study by Zhou *et al*. (2020)[17] found 70.96% of cases who were asymptomatic on the identification date went on to develop disease and were defined as Asymptomatic Patients in the Incubation period (APIs), where the median CT value of patients remained asymptomatic (APs: Asymptomatic Patients) was significantly higher than that of APIs.

This study also found a significant relationship between gender, age, comorbidities, and radiological findings with the severity of clinical symptoms. The percentage of moderate and severe group was higher in male and radiological features of pneumonia. In contrast, the percentage of asymptomatic and mild group was higher in female and non-pneumonia radiological finding. Furthermore, the patients in severe group were older (≥ 60 years) and had at least one comorbidity than those in asymptomatic, mild, and moderate groups. Liu *et al*. (2020)[8] also reported older patients were more easily infected with SARS-CoV-2 and develop severe symptom. In terms of gender, the results we obtained was consistent with the study from Jin *et al*. (2020)[18] which reported men tends to be more severe. This is probably related to the protection function of X chromosome and estrogen receptors which had an important part in the mechanism of immune system[13]. Moreover, comorbidities (including hypertension, cardiovascular disease, and lung disease), smoking and drinking alcohol are more frequent in men[19].

However, based on multinomial logistic regression analysis, only radiological features of pneumonia, comorbidities, and CT value were significantly related to the severity of clinical symptom. Rico *et al* (2021)[20] also found that sex did not pertain to COVID-19. SARS-CoV-2 is more likely to infect older people with comorbidities due to the decrease of patient's immune functions[21]. In contrast, Wang *et al*. (2020) who determined the presence of a radiologic abnormality, found there was no difference in gender in terms of ICU admission. However, they reported that ICU-admitted patients were older and had several comorbidities than non-ICU patients. This showed that age and comorbidities can be risk factors for poor outcome[22]. On Computerized tomography (CT) scan, Guan *et al*. (2020) reported normal chest CT scan was seen even in severe patients[23]. The sensitivity and specificity of CT scan was low, and it should be considered as an additional diagnostic tool, especially for symptomatic patients, and it was necessary to be verified with clinical symptom and viral RNA detection[6,24].

This study has several limitations. First, we did not follow up the clinical progression of the disease and CT value during hospitalization, thus the outcome and viral shedding were not known. Second, the distribution of sample number in each group of severity is very different, which could lead to an unbalanced distribution. Last, the CT value results were highly dependent on several technical matters, including the quality of collected samples, the amount of genetic material contained in the sample, and the extraction method performed.

CONCLUSION

The CT value of RT-PCR assays has significant negative correlation with the severity of clinical symptoms in all patients with different clinical symptom severity at admission. Adjusted by other variables, CT value still has a significant association with severity of COVID 19 symptom.

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REFERENCES

1) Huang C, Wang Y, Li X, Ren L, Zhao J. Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuhan, China. *Lancet*. 2020; 395: 497-506.

2) Huang Y, Chen S, Yang Z, *et al*. SARS-CoV-2 Viral Load in Clinical Samples from Critically Ill Patients. *American Journal of Respiratory and Critical Care Medicine*. 2020; 201(11): 1435-38.

3) Sugihantono A, Erlina B, Erlang S, *et al*. Epidemiologi. In: Pedoman Pencegahan dan Pengendalian *Corona Virus Disease* (COVID-19) Revisi Ke-5. Jakarta, Kementrian Kesehatan RI; 2020: 19-21.

4) World Health Organization. WHO Coronavirus (COVID-19) Dashboard. Published 2020. Available at: *https://covid-19.who.int/*.

5) Xu T, Chen C, Zhu Z, *et al*. Clinical Features and Dynamics of Viral Load in Imported and Non-Imported Patients with COVID-19. *International Journal of Infectious Diseases*. 2020; 94: 68-71.

6) Karahasan Yagci A, Sarinoglu RC, Bilgin H, *et al*. Relationship of the Cycle Threshold Values of SARS-CoV-2 Polymerase Chain Reaction and Total Severity Score of Computerized Tomography in Patients with COVID 19. *International Journal of Infectious Diseases*. 2020; 101: 160-6

7) Li T, Wang L, Wang H, *et al*. Characteristics of Laboratory Indexes in COVID-19

Patients with Non-Severe Symptoms in Hefei City, China: Diagnostic Value in Organ Injuries. *European Journal of Clinical Microbiology & Infectious Diseases*. 2020; 39: 2447-55.

8) Liu Y, Yan LM, Wan L, *et al*. Viral Dynamics in Mild and Severe Cases of COVID-19. *Lancet*. 2020; 20(6): 656-7.

9) Yu X, Sun S, Shi Y, *et al*. SARS-CoV-2 Viral Load in Pputum Correlates with Risk of COVID-19 Progression. *Crit Care*. 2020; 24: 1-4.

10) Zheng S, Fan J, Yu F, *et al*. Viral load Dynamics and Disease Severity in Patients Infected with SARS-CoV-2 in Zhejiang Province, China, January-March 2020: Retrospective Cohort Study. *BMJ*. 2020; 369: 1-8.

11) Shah S, Singhal T, Davar N, Thakkar P. Initial Observations with Molecular Testing for COVID-19 in a Private Hospital in Mumbai, India. *Indian Journal of Pediatrics*. 2020; 87(7): 555.

12) Zhang J, Wang X, Jia X, *et al*. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. *Clinical Microbiology and Infection*. 2020; 26: 767-72.

13) Karyono DR, Wicaksana AL. Curr]ent Prevalence, Characteristics, and Comorbidities of Patients with COVID-19 in Indonesia. *Journal of Community Empowerment for Health*. 2020; 3(2): 77-84.

14) O'Brien J, Du KY, Peng C. Incidence, Clinical Features, and Outcomes of COVID-19 in Canada: Impact of Sex and Age. *Journal of Ovarian Research*. 2020; 13: 1-12.

15) Zou L, Ruan F, Huang M, *et al*. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *New England Journal of Medicine*. 2020; 382(12): 1175-7.

16) Chu CM, Poon LLM, Cheng VCC, *et al*. Initial Viral Load and The Outcomes of SARS. *CMAJ*. 2004; 171(11): 1349-52.

17) Zhou R, Li F, Chen F, *et al*. Viral Dynamics in Asymptomatic Patients with COVID-19. *International Journal of Infectious Diseases*. 2020; 96: 288-90.

18) Jin JM, Bai P, He W, *et al*. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. *Frontiers in Public Health*. 2020; 8: 1-6.

19) Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 Patophysiology: A Review. *Clinical Immunology*. 2020: 1-7.

20) Sitorus RJ, Wathan I, Ridwan H, *et al*. Transmission Dynamics of Novel Coronavirus ─ SARS-CoV-2 in South Sumatera, Indonesia. *Clinical Epidemiology and Global Health*. 2021; 11: 1-4.

21) Chen N, Zhou M, Dong X, *et al*. Epidemiological and Clinical Characteristics of 99 Cases of 2019 Novel Coronavirus Pneumonia in Wuhan, China: a Descriptive Study. *Lancet*. 2020; 395: 507-13.

22) Wang D, Hu B, Hu C, *et al*. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *Journal of the American Medical Association*. 2020; 323(11): 1061-9.

23) Guan W, Ni Z, Hu Y, *et al*. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*. 2020; 382: 1708-20.

24) Grace C. Manifestasi Klinis dan Perjalanan Penyakit pada Pasien Covid-19. *Majority*. 2020; 9: 49-55.