Cycle Threshold Value and Absolute Lymphocyte Count Relationship with Severity of COVID-19

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Abstract. Coronavirus Disease 2019 (COVID-19) is a case that spread to many countries, including Indonesia, due to of SARS-CoV-2 transmission. West Sumatra, especially Padang city, is one of the highest spread of COVID-19 cases in Indonesia. This study aimed to examine the relation between Ct value and absolute lymphocyte count with the severity of COVID-19. The type of this research was analytic observational with the cross sectional study. We collected 106 patients data in Dr. M. Djamil Padang hospital from moderate to critical severity from June-August 2021. The data were collected and processed using convenient sampling which was then tested using Spearman correlation, One Way ANOVA, and Kruskal-Wallis tests. The results showed that most patients with confirmed COVID-19 were at the age of 56 to 65 years (30.2%), were women (51.9%), and had hypertension comorbidity (27.6%). We found that the median (IQR) of the Ct value is 23.5(10) and the mean ± SD of absolute lymphocyte count is 1213±56. There was a relation between Ct value and severity of COVID-19 (p=0.019), absolute lymphocyte count and severity of COVID-19 (p<0.01), and weak negative correlation of Ct value and absolute lymphocyte count (p=0.040, ρ=-0.200). Increasing viral load may elicit higher immune response clinically in moderate to critical COVID-19 patients.

Keywords: Absolute Lymphocyte Count, COVID-19, Ct Value, SARS-Cov-2

1. INTRODUCTION

At the end of December 2019, there was an outbreak with symptoms of fever, dry cough, fatigue, and other symptoms in Wuhan, Hubei, China. This outbreak is also called the outbreak of the novel coronavirus SARS-CoV-2 (Coronavirus disease 2019; previously 2019-nCoV). This outbreak has spread to various countries. On March 2nd, 2020, the first case of COVID-19 was confirmed in Indonesia which became one of the countries with the highest cumulative cases of COVID-19 in the world. The peak of the active cases of COVID-19 in Indonesia occurred in June, July, and August 2021 with the highest increase in the third week of July with 166,221 cases. Mortality cases in August are still high and need more attention in handling COVID-19. High mobility, breaking the health protocols, and the entry of the delta variant into Indonesia brought high cases of COVID-19 in Indonesia.

Good treatment can help reduce the severity of COVID-19 cases. Handling COVID-19 can start with a good screening process, accurate diagnostics, and the best monitoring of confirmed cases of COVID-19 that are being treated in hospitals. The diagnostic process for COVID-19 includes carrying out a PCR swab examination and observing it through the Cycle threshold (Ct) value. Research in Brazil stated that COVID-19 patients with high viral loads/low Ct values have a higher mortality rate. Approximately

85% of COVID-19 patientswith severe symptoms developed lymphopenia. Lymphopenia is a condition witha low number of lymphocytes in the blood (<1500 cells/mm3), especially NK andCT cells that often occurs in elderly patients over 60 years of age and in patients who need intensive care with a drastic decrease in CD8+.

Symptoms of COVID-19 have a certain level or degree, such as asymptomatic, mild symptoms, moderate symptoms, severe symptoms, and critical. COVID-19 patients will usually be hospitalized with moderate, severe,and critical symptom levels. This study aimed to give more knowledge about COVID-19, because there are so many things that still unknown about this virus, especially the process of monitoring COVID-19 patients, so with this research, the handling process of COVID-19 cases can be better and more accurate.

2. METHODS

This study was a cross-sectional analytical study, conducted at Dr. M. Djamil Padang hospital with a convenient sampling method. The subject of research were COVID-19 patients with moderate, severe, and critical severity admitted in June-August 2021. The data were collected from the medical record in Dr. M. Djamil Padang hospital that were approved by the ethical committee (No: I.B.02.02/5.7/305/2022). The inclution data of this research was the first examination of Ct value when the patient was admitted, an absolute lymphocyte count that had a minimum interval of <48 hours from the first examination of the Ct value, and moderate to critical clinical symptoms. The exclude data was Patients with confirmed COVID-19 at M. Djamil Hospital Padang in the period June-August 2021 who have comorbidities with clinical manifestations comparing COVID-19 (asthma, lung cancer, bacterial pneumonia, fungal pneumonia, SARS/MERS, cardiogenic pulmonary edema/heart failure) and diseases with abnormalities in the number of lymphocyte cells.

The sample size was obtained by using the Lemeshow formula $n = \frac{z^2 p\ (1-p)}{d^2} \rightarrow n = \frac{(1.96)^2 0.5\ (1-0.5)}{0.01} = 96$ samples. We used the drop out data 10% so the last sample size was 106 samples. The normalitytest was conducted to determine the distribution of the data and data were analyzed using One Way ANOVA for absolute lymphocyte count and severity of COVID-19, Kruskal-Wallis for Ct value and severity COVID-19, Spearman correlation for Ct value and absolute lymphocyte count. The data of Ct value used median (Interquartil range/IQR) because the data were not normally distributed, whereas the absolute lymphocyte count data used mean ± Standard Deviation(SD) because the data were

normally distributed. Statistical analysis was processed using IBM SPSS statistics v.25.

3. RESULTS

In 106 COVID-19 patients at Dr. M. Djamil Padang hospital that were included, predominantly 56-65 years old, male, hypertension comorbidity, moderate severity. Some of COVID-19 patients at Dr. M. Djamil Padang hospital had more than one comorbidity. The median(IQR) of Ct value were 23,5 and 10. Mean ± SD of absolute lymphocyte count was 1213±56. This data shown in table 1.

Ct value and severity of COVID-19 were not normal distribution. There was significant between these variables because of p value < 0,05. This data data shown in figure 1. There was significant between absolute lymphocyte count andseverity of COVID-19 shown in figure 2, and these variables were normal distribution. Ct value and absolute lymphocyte count were tested and there werevery weak significant. This significant data shown in figure 3.

Table 1. Clinical Characteristics, Ct value, Absolute Lymphocyte Count and Severity of COVID-19 in Dr. M. Djamil Padang Hospital (n=106)

Variables	Median (IQR)	Mean ± SD	(n)	(%)
Age (Year)	55(24)			
18-25			8	7.5
26-35			16	15
36-45			9	8.5
46-55			23	21.8
56-65			32	30.2
>65			18	17
Sex				
Male			51	48.1
Female			55	51.9
Comorbidity				
Hypertension			32	27.6
Diabetes mellitus			23	19.7
Liver disease			16	7.8
Kidney disease			13	11.2
Heart disease			9	7.8
CAP			8	6.9
Dyspepsia			4	3.4
Asthma			5	4.3
Malignancy			3	2.6
Malaria			1	0.9
Urinary tract infection			1	0.9
Pleural effusion			1	0.9
Severity of COVID-19				

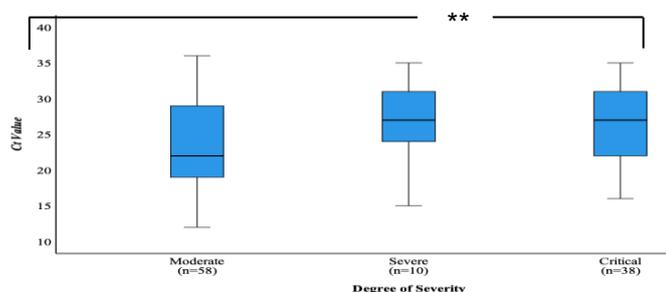|				
Moderate			58	54.7
Severe			10	9.4
Critical			38	35.9
Ct value		23.5(10)		
Absolute Lymphocyte Count		1213±651		

Figure 1. Boxplot statistical test of Ct valueand degrees of severity of COVID-19.
**p<0.01 difference between moderate and critical groups (Kruskal-Wallis test followed by Mann Whitney Post Hoc test)
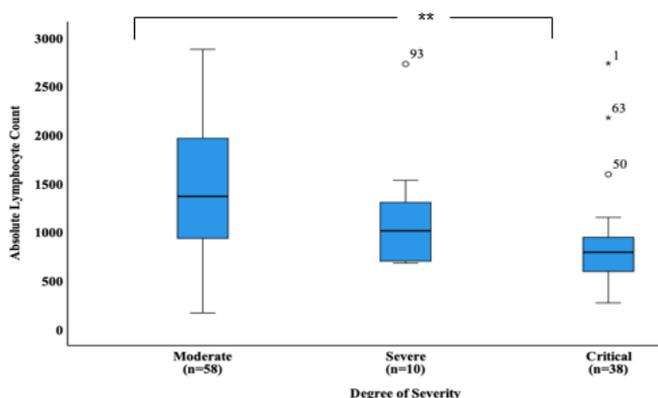

Figure 2. Boxplot statistical test absolute lymphocyte count and degrees of severity of COVID-19. **p<0.01 difference between moderate and critical groups (One WayANOVA test followed by Bonferroni Post Hoc test)
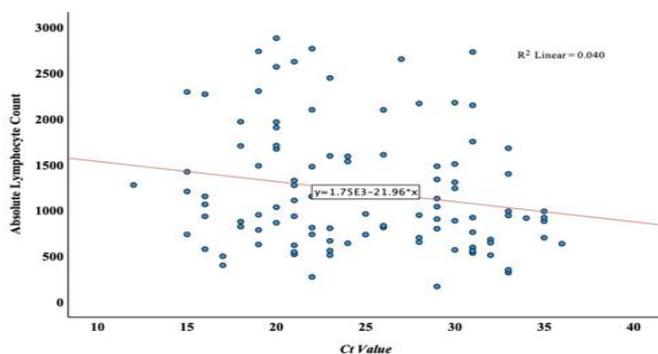

Figure 3. Scatter plot statistical test for the relationship between Ct value and absolute lymphocyte count of COVID-19 patients using the Spearman test (p=0.040, ρ=-0.200)

4. DISCUSSION

The description of clinical characteristics in this study was dominated bythe age of 56-65 years (late elderly), this could occur due to old patients experiencing degeneration of body functions and the body's immune system so that they will be more susceptible to infection, especially COVID-19 infection. The negligence factor in maintaining or implementing health protocols is also animportant factor in the spread of infection in old age which makes it easier.

The gender in this study was dominated by the female sex although the numbers between the male and female sexes were not much different. Some researchers said that women are more vulnerable to stress in dealing with a pandemic so their body's immunity will also decrease and they will be easily infected. Men are also easily infected with COVID-19 due to more activities outside the home than women, smoking habits, and a low level of knowledge compared to women.

Hypertension was the most common comorbidity in this study.Hypertension is still the most common comorbid in Indonesia, which is 50.4%. This hypertension condition will exacerbate the condition of COVID-19 patients. Data on Ct values and the degree of severity of COVID-19 vary between hospitals, this indicates that the output of COVID-19 infection varies foreach patient.

Lymphocytopenia can occur due to an IL-6 inflammatory cytokine storm.It has been proven that there is a significant relationship between IL-6 serum levels in COVID-19 patients and decreased T-cell activity, so the process of inducing apoptosis in lymphocytes becomes larger, decreasing the activity and the function of T lymphocyte cells. A low lymphocyte count can also be causedby elderly patients who tend to have a low absolute lymphocyte count. A study also revealed that activation of CD4+ T lymphocyte cells is more dominant in COVID-19 patients with severe clinical conditions, comorbidity in the patient also affects the work of lymphocyte cells which will be increasingly difficult to maintain immunity. The study also stated that lymphocytosis in COVID-19 patients could occur in B-cell lymphocytes that produce antibodies.

Disease progression or a long viral clearance process will have a more severe disease impact. The lower the Ct value detected at the start of the COVID-19 infection, the more severe and long the disease will progress. The patient's condition can lead to ARDS, cytokine storm, multiorgan failure, and death. A retrospective study in Mumbai, India in All patients with RT-qPCR diagnosed COVID 19 illness and for whom Ct values were available pointed to the fact that it was difficult to see a correlation between the Ct value and

the severity of COVID-19. In their study, COVID-19 patients with a mild degree of severity had a low Ct value (high viral load) and were also tested earlier than COVID-19 patients with a severe degree of severity. This made it difficult for them to draw definite conclusions about the relationship between Ct values and the degree of severity of COVID-19 due to the many ambiguities in this study.

Research in Bethesda Hospital, Yogyakarta in all COVID-19 patients stated that the degree of severity of critical COVID-19 had the lowest number of lymphocytes among other degrees of severity. Lowlymphocytes in patients with severe severity of COVID-19 – critical are causedby several factors, namely inflammatory cytokine storms, the process of viral clearance. for a long time causing a decrease in function and impaired expansionof T cell lymphocytes.

Research in COVID-19 patients in First Hospital of Jiaxing, China stated tha there is a positivecorrelation between Ct value and lymphocyte count. A low Ct value tends to increase C-reactive protein (CRP). CRP is a specific marker in the acute phase ofthe systemic inflammatory response, this CRP will promote the movement of neutrophils and macrophages, inhibiting the mixed reaction of lymphocytes. Thisconcluded that the Ct value was positively correlated with the severity of COVID-19. Researchin Nayarit, Mexico in outpatients found thatthere was a negative correlation, the lower the Ct value, the higher the absolute lymphocyte count (and vice versa). A low Ct value is associated with a high riskof severe infection so that the immune system's resistance processes such as lymphocyte cells in fighting viruses will be higher so the number of lymphocytesto be detected will be high.

This study had limitation that too simple to see the relationships between Ct value, absolute lymphocyte count and the severity of COVID-19 itself. The research design was cross-sectional by looking at medical record data which only focused on patient characteristics according to the first Ct value examination and absolute lymphocyte count at RSUP Dr. M. Djamil Padang and only provides an overview, does not really explain how the progress of COVID-19 and how the Ct value is detected in patients, because in reality this COVID-19 infectious disease on average has a fairly long disease course and certainly many factors. affecting infectivity in patients. This will certainly affect the patient's viral load / Ct value, but in this study other factors that influence the progression of the COVID-19 disease are not explained, but it is important for medical staff to know more about the Ct value and its relationship with the degree of severity. COVID-19 so that the dynamics of COVID-19 disease can be understood.

This study also had many shortcomings in terms of data collection, the research focus which only looked at clinical characteristics and the patient's lymphocyte count on one particular day made this research seem less robust. Research like this is better done by following the progress of the patient so that the factors involved in determining the severity of COVID-19 become more widely known and a clearer consideration is made of whether lymphocytes are related to the severity of COVID-19. The weakness in this study also lies in the process of taking Ct value data which does not vary with the initial appearance of COVID-19 symptoms so that the data obtained is very diverse.

5. CONCLUSION

In conclusion, there is a relationship between Ct value and severity of COVID-19, absolute lymphocyte count and severity of COVID-19, weak relation between Ct value and absolute lymphocyte count.

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