Soluble Cluster of Differentiation 25 (sCD25) as a Predictor of Mortality of COVID-19 Patients in Surabaya, Indonesia

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Abstract

The purpose of this study was to analyze and determine the cut-off level of sCD25 as a predictor of mortality in COVID-19 patients. In an observational analytic study with a prospective cohort design, the study population was COVID-19 patients who were hospitalized at RIK RSUD Dr. Soetomo Surabaya for the period July 2020-December 2020. Sampling was taken by consecutive sampling, divided into two groups, mild-moderate and severe-critical groups. The examination of sCD25 levels in both groups was carried out on day-0 and day-6 of hospitalization using the sandwich ELISA method. The paired group statistical analysis used the Wilcoxon range test, the unpaired group used the Mann Withney U test. ROC curve analysis to determine the cut off level of sCD25 as a predictor of mortality. There were a total of 83 study patients consisting of 36 patients in the mild-moderate group, 47 patients in the severe-critical group. There was a difference in sCD25 levels between mild-moderate COVID-19 patients who were treated on day-6 compared to day-0, whereas in the severe-critical group there was no difference in sCD25 levels. There was a difference in sCD25 levels in COVID-19 patients between the mild-moderate group by severe-critical. The level of sCD25 with a cut off of 3.14 ng/mL (AUC 0.719, p = 0.001) can be used as a predictor of mortality in COVID-19 patients with a sensitivity of 96.2%, a specificity of 47.4%. Levels of sCD25 >3.14 ng/mL can be used as a predictor of mortality in COVID-19 patients

Keywords: *sCD25, COVID-19, severe-critical, mild-moderate, mortality*

Introduction

COVID-19 is an infectious disease caused by SARS-CoV-2. SARS-CoV-2 is a new type of Coronavirus that has never been previously identified in humans. This disease was identified starting with the emergence of a pneumonia case of unknown etiology in Wuhan, China at the end of December 2019[1,2,3]. COVID-19 spreads to various countries in a short time so that on March 11, 2020, the WHO determined this incident to be a Pandemic[2,4,5]. Indonesia reported its first case on 2 March 2020 then reported 70,736 confirmed COVID-19 cases with 3,417 deaths (CFR 4.8%) as of 9 July 2020[3].

COVID-19 gives varying symptoms, about 80% of sufferers have mild or asymptomatic infections, 15% have severe infections and need oxygen, and 5% are in critical condition and need a ventilator[6]. It is estimated that 10-15% of mild cases progress to severe, and 15-20% of severe cases become critical. The CFR among critical cases was 49% and patients with older age and comorbids are factors that can increase the mortality of COVID-19 patients[7]. The severity of the disease in COVID-19 patients generally occurs 1 week after the appearance of symptoms[8,9].

Hyperinflammation or cytokine storms are known to play an important role in the severity process of the disease[10]. Clinicians must be aware of this potential for deterioration so that it can reduce the mortality rate for COVID-19 patients.

Soluble Cluster of Differentiation 25 (sCD25) or also known as Soluble Interleukin-2 receptor α (sIL-2Rα) is a marker of inflammation and a diagnostic marker of immune system activation[11]. COVID-19 patients develop a hyperinflammatory syndrome that has similarities with other hyperinflammatory disorders[12], so it should be expected that the sCD25 marker can assess the inflammatory process and can be used as a predictor of mortality in COVID-19 patients. Soluble CD25 (sCD25) results from cleaving the CD25 receptor when T cells, B cells and dendritic cells are activated. The CD25 receptor is cut from the surface of the cell membrane by a protease enzyme, namely matrix metalloproteinase-9 (MMP-9) produced by T cells, macrophages, and dendritic cells induced by inflammatory conditions. The cutting of CD25 to form soluble CD25 (sCD25) can also reflect IL-2 activity attached to its receptors as well as the proliferation and activation of T lymphocytes[13,14].

Based on the above explanation, the researchers wanted to examine whether sCD25 levels could be used as a predictor of mortality in COVID-19 patients associated with immune cell activation and inflammatory processes that occur in COVID-19 patients, so that they can be used to predict mortality in COVID-19 patients.

Materials and Methods

This type of study was an observational analytic study with a prospective cohort design. The study population was COVID-19 patients who were hospitalization in Ruang Isolasi Khusus (RIK) Dr. Soetomo Surabaya for the period July–December 2020. Study patients were taken from the study population who met the inclusion and exclusion criteria using consecutive sampling technique. Based on the severity level, it is divided into two groups, namely the mild-moderate and severe-critical groups. The examination of sCD25 levels was carried out on day-0 and day-6 of hospitalization. Serum samples were stored at -80^{o}C pending examination. Examination of sCD25 levels used Bioassay Technology Laboratory reagent with the sandwich ELISA method. Paired group statistical analysis used the Wilcoxon sign rank test method, the unpaired group used method mann withney u test. ROC curve analysis was used to determine the cut-off level of sCD25 on day-0 of hospitalization as a predictor of mortality within 30 days of COVID-19 patients.

Results and Discussion

There was a total of 83 study patients who met the inclusion and exclusion criteria consisting of 28 female patients (33.7%) and 55 male patients (63.3%). The mild-moderate group consisted of 36 patients and the severe-critical group of 47 patients. Based on gender, men were more in the severe-critical group, while women were more in the mild-moderate group (Table 1). COVID-19 shows different case fatality between men (2.8%) and women (1.7%), this is associated with ACE2 expression. ACE2 activity in men is lower than women because estrogen triggers an increase in ACE2 expression more than androgens[15].

In the severe-critical group, it was found that the median age of patients was older than the mild-moderate group and comorbids were more common in the severe-critical group (Table 1). Age >65 years is a factor associated with the severity and mortality of COVID-19 patients[15,16,17]. This is related to the function of the immune system and organ function. The function of T cells and B cells decreases with age[18,19,20]. The presence of comorbidities is known to increase the case fatality rate (CFR) and provide a poor prognosis in COVID-19 patients[21,22]. In this study, there were no significant differences based on comorbid (Table 1).

Table 1. Characteristics of research subjects based on gender, age and co-morbidity in the mild-moderate and severe-critical groups.

Variable	Mild – Moderate (N = 36) n (%)	Severe – Critical (N = 47) n (%)	P value
Gender			
Male n (%)	17 (31.5%)	37 (68.5%)	0.005
Female n (%)	19 (65.5%)	10 (34.5%)	
Age	51 (24-68)*	53 (25-72)*	0.076
Obesity			
N n (%)	27 (40.9%)	39 (59.1%)	0.419
Y n (%)	9 (52.9%)	8 (47.1%)	
Asthma			
N n (%)	34 (42.5%)	46 (57.5%)	0.576
Y n (%)	2 (66.7%)	1 (33.3%)	
Hypertension			
N n (%)	30 (47.6%)	33 (52.4%)	0.202
Y n (%)	6 (30.0%)	14 (70.0%)	
Diabetes Mellitus			
N n (%)	27 (49.1%)	28 (50.9%)	0.165
Y n (%)	9 (32.1%)	19 (67.9%)	

*Median (Min-Max). Description: Y: Yes, N = No,

In this study, the levels of sCD25 were not significantly different between the mild-moderate and severe-critical groups based on sex, age and comorbid obesity, asthma, hypertension. Significant differences in sCD25 levels were only found in patients with comorbid diabetes mellitus in the two groups (p = 0.005; p = 0.018) (Table 2). Increased levels of sCD25 may be related to the chronic inflammatory process that occurs in diabetes mellitus called chronic low-grade inflammation, which is characterized by the production of cytokines, chemokines, and adipokines[19].

Table 2. Levels of sCD25 by age, sex and comorbidity in the mild-moderate and severe-critical groups.

Characteristics of sCD25	Group			
	Mild - Moderate		Severe - Critical	
	Median (Min-Max)	p Value	Median (Min-Max)	p Value
Age				
≤ 50	3.1 (1.7-9.9)	0.129	3.5 (0.4-24.1)	0.121
> 50	2.7 (0.9-9.9)		5.3 (0.6-18.4)	
Gender				
Male	3.1 (0.9-7.4)	0.925	4.8 (0.4 - 24.1)	0.404
Female	2.7 (1.1-9.9)		4.7 (3.5 - 13.3)	
Obesity				
N	2.9 (0.9-9.9)	0.886	3.8 (0.4 - 24.1)	0.132
Y	2.7 (2.2-8.6)		6.0 (3.5 - 17.1)	
Asthma				
N	2.8 (0.9-9.9)	0.133	4.6 (0.4-24.1)	1.000
Y	6.1 (4.8-7.4)		-	
Hypertension				
N	3.1 (0.9-9.9)	0.123	5.0 (0.4-24.1)	0.383
Y	2,6 (1.2-4.3)		3.7 (2.2-13.3)	
Diabetes Mellitus				
N	3.1 (1.7-9.9)	0.005	3.5 (0.4-24.1)	0.018
Y	2.5 (0.9-9.9)		6.3 (3.0-18.4)	

Information: Y= Yes, N= No

In the mild-moderate groups, there was a significant difference in sCD25 levels between day-6 and day-0 of hospitalization (p = 0.016) (Table 3). Of a total of 36 patients in the mild-moderate group, 23 patients (64%) had decreased levels of sCD25 and 13 patients (36%) had an increase in sCD25. The level of sCD25 decreased from a median of 2.92 ng/mL on day-0 to 2.80 ng/mL on day-6 of hospitalization (Table 3). This can indicate that in patients there has been a decrease in the activation of immune cells, especially cells that express interleukin-2 receptor (IL-2R), so that the level of sCD25 in the blood decreases. In the severe-critical group, there was no significant difference in sCD25 levels between day-6 and day-0 of hospitalization (Table 3). This is because of a total of 47 patients in the severe-critical group, 27 patients (57%) experienced a decrease in sCD25 levels and 20 patients (43%) experienced an increase in sCD25 levels so that their sCD25 levels were not significantly different.

There were significant differences in sCD25 levels on day-0 and day-6 between the mild-moderate group and the severe-critical group (p = 0.001; 0.004). The median level of sCD25 for the critical severe group tended to be higher when compared to the median for the mild-moderate group both on day-0 and day-6 of hospitalization (Table 3). This is consistent with a number of studies where sCD25 levels are positively correlated with severity[10,20,21,22]. In severe degrees of COVID-19, the increase in sCD25 levels is thought to be due to inflammation which increases the action of a proteolytic enzyme, namely MMP-9 which cuts sCD25 on the cell surface[23].

Based on the patient's outcome of the severe-critical groups, 21 patients recovered and 26 patients died. There was no significant difference in sCD25 levels between 6 and 0 days of hospitalization in the critically severe group with outcomes of recovery or death (Table 3). In patients who recovered, the median sCD25 decreased, while in patients who died, the median sCD25 increased although it was not statistically significant. A longitudinal analytical study identified innate and adaptive immune response patterns severe COVID-19. In severe patients there is a delay in the immune response or the course of the immune response appears to be protracted and irregular. In the severe COVID-19 cohort, changes in immune response occurred less dynamically and varied for 15 days after hospitalization. This may explain why in the severe-critical group there was no significant change in sCD25 levels on the 6th day of hospitalization[23]. It takes a longer observation time to get a significant change in sCD25 levels.

Table 3. sCD25 levels in COVID-19 patients by group on day-0 and day-6 of hospitalization.

Variable	Group	Days to-	Median (Min-Max)	p-value
sCD25 (ng/mL)	Mild - Moderate	0	2.92 (0.94-9.91)	0.016
		6	2.80 (0.81-8.84)	
	Severe - Critical	0	4.78 (0.24-24.07)	0.167
		6	4.09 (1.03-23.55)	
	Mild - Moderate	0	2.92 (0.94-9.91)	0.001
	Severe - Critical	0	4.78 (0.40-24.07)	
	Mild - Moderate	6	2.80 (0.81-8.84)	0.004
	Severe - Critical	6	4.09 (1.03-23.55)	
	Severe - Critical (Outcome Recovery)	0	3.58 (0.4-24.07)	0.198
		6	3.17 (1.03-23.55)	
	Severe - Critical (Outcome Deaths)	0	5.30 (2.17-18.36)	0.477
		6	5.46 (1.06-23.55)	

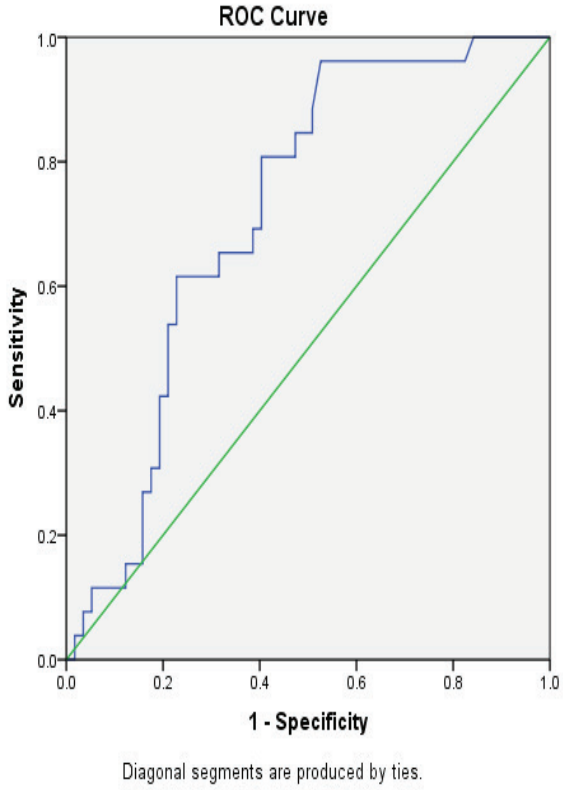

Figure 1. Receiver Operating Characteristics (ROC) curve to determine the cut-off level of sCD25 as a predictor of mortality in COVID-19 patients.

ROC analysis was performed to determine the cut off of sCD25 levels on day-0 to predict mortality within 30 days of COVID-19 patients. The mortality analysis was carried out on all patients (mild-moderate, severe-critical) as many as 83 people with a death outcome of 26 patients and 57 patients recovered. The results of the ROC analysis showed that the cut off level of sCD25 was 3.14 ng/mL (Area Under Curve 0.719, p = 0.001) with a sensitivity value of 96.2% and a specificity of 47.4% (Figure 1). With this cut off, clinicians are expected to be more aware of the potential worsening of COVID-19 patients so that they can reduce the mortality rate for COVID-19 patients. Low specificity can indicate that sCD25 can be said to be not a specific marker in predicting mortality in COVID-19 patients, combination with other markers is needed to increase its specificity.

This study has limitations, that there is no complete data regarding when the patient was infected so that the onset of symptoms cannot be ascertained and may can affect the level of sCD25 at the time of examination. Observation and measurement of sCD25 levels in the severe-critical group were carried out more than 6th day of hospitalization, a longer treatment time was needed in order to get a picture of the changes in sCD25 after the 6th day of hospitalization.

Conclusion

In summary, there was a difference in sCD25 levels between the mild-moderate group of COVID-19 patients who were hospitalized on day-6 and day-0, whereas in the severe-critical group there was no difference in sCD25 levels. There was a difference in the sCD25 of COVID-19 patients between the mild-

moderate and severe-critical groups. The sCD25 level at day-0 with a cut off >3.14 ng/mL can be used as a predictor of mortality within 30 days of COVID-19 patients with a sensitivity of 96.2%, a specificity of 47.4%.

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