Comparison of Serum Interleukin-6 Levels between Survivor and Non-Survivor of Hospitalized COVID-19 Patients

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Abstract

Objective: Coronavirus Disease-19 (COVID-19) causes symptoms that vary from asymptomatic to severe. The basis of the more severe condition is hypothesized to be an exaggerated immune response called cytokine release syndrome (CRS). One of the cytokines that play a major role is interleukin-6 (IL-6). An elevated level of IL-6 correlates with the amount of RNA SARS-CoV-2 in the blood (viremia) which is associated with severity and occurrence of complications. This study aims to compare serum IL-6 between the survivor and non-survivor of hospitalized COVID-19 patients.

Methods: Eligible subjects were the confirmed COVID-19 adult patients who had their IL-6 serum levels measured before starting anti-IL-6 therapy. Data were collected retrospectively at Dr. Hasan Sadikin General Hospital from November 2020 to April 2021. A statistical comparison test was performed to compare the IL-6 serum levels of non-survivor and survivor groups.

Results: There were 136 COVID-19 patients who met the inclusion criteria, with 89 survivors and 47 non-survivors. The Mann-Whitney comparison test revealed that the non-survivor group had higher median IL-6 levels than the survivor group (41.4 vs 10.8 pg/ml, $p<0.001$).

Conclusion: IL-6 levels were significantly higher in the non-survivor than the survivor group of hospitalized COVID-19 patients. The findings of this study can serve as a starting point for larger and more in-depth studies on the role of IL-6 in COVID-19.

Keywords: Coronavirus Disease-19, Interleukin-6, non-survivor, survivor

Introduction

The World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) as a public health emergency on March 11, 2020. Until the present moment, the disease has affected over 219 million people worldwide and resulted in the deaths of 4.550.000 people.[1] Since the first COVID-19 case was reported in

Indonesia on March 2, 2020, more than 4 million people have been confirmed to have acquired COVID-19, with a mortality rate of 3.3% compared to a global mortality rate of 2.7%.[2]

The majority of COVID-19 patients have an asymptomatic or mild influenza-like course, while a minority have more severe symptoms such as severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, and death.[3] The underlying mechanism causing severe COVID-19 complications such as ARDS and multi-organ failure is thought to be an overactive immune response known as cytokine release syndrome (CRS). An increase in inflammatory mediators promotes CRS and is linked to a poor prognosis in COVID-19 patients.[4,5] Several inflammatory mediators that are increased in CRS of COVID-19 cases include C-reactive protein (CRP), procalcitonin (PCT), ferritin, interleukin-1 (IL-1), interleukin-2R (IL-2R), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor (TNF)-alpha.[4–6] Among these inflammatory mediators, the increase of serum IL-6 concentration has been reported to exceed the other mediators and may reach 1.000 times the normal value.[6]

Interleukin-6 (IL-6) is secreted by stromal cells by the stimulation of the pro-inflammatory cytokines, particularly interleukin-1 (IL-1) and TNF-α. At the start of the infection, lung macrophages produced IL-6 in response to toll-like receptor (TLR) stimulation. Because the increase in IL-6 occurred before acute lung injury in COVID-19, it is used as a marker for severe disease. However, there is debate over whether excessive IL-6 synthesis is the true cause of ARDS in COVID-19 or merely an epiphenomenon. According to the dominant theory, excessive IL-6 expression contributes significantly to cytokine storm and causes lung injury as well as ARDS. This inflammatory mediator can also increase lung capillary permeability, promote the development of ARDS, and initiate a coagulation pathway that leads to the formation of microthrombus in the lung circulation in addition to the occurrence of thrombotic events.[7]

The amount of SARS-CoV-2 RNA in the blood (viremia) correlates with an increase in serum IL-6 levels. Furthermore, viremia in COVID-19 is linked to severe disease, ARDS, and multi-organ failure.[8,9] Several studies have linked elevated serum IL-6 levels to an increased risk of death in COVID-19 patients.[10–12] In the pandemic situation, the discovery of the laboratory markers that help to predict COVID-19 severity is important because they affect resource allocation demand, for example, ventilator machines. Several global research on IL-6 concentration increase has been conducted, but no local research has been done at dr. Hasan Sadikin Central General Hospital (RSHS). Therefore, this study aims to compare the serum IL-6 concentration of COVID-19 survivors and non-survivor in dr, Hasan Sadikin Hospital from November 2020 until April 2021.

Methods

The design of this study was observational, descriptive, and comparative. The data were collected retrospectively by the cross-sectional method. The IL-6 level was evaluated with the chemiluminescent immunoassay (CLIA) procedure using Advia Centaur, and secondary data was collected from COVID-19 patients' medical records during their hospital stay in dr. Hasan Sadikin General Hospital, Bandung. The subjects of this study were adult patients who were confirmed as positive result of COVID-19 in dr. Hasan Sadikin General Hospital, Bandung on period November 2020 until February 2021. The inclusion criteria of this study were adult patients aged ≥ 18 years old who were confirmed as COVID-19 case, hospitalized, and was examined for IL-6 level before having anti-IL-6 therapy (tocilizumab). The exclusion criteria include (1) pregnant women, (2) subjects with systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis, and (3) subjects having a chronic disease with the consumption of immunosuppressant drugs. The survivor group was hospitalized COVID-19 patients who were discharged in healthy or improved condition. On the contrary, the non-survivor group was patients who passed away during the hospital stay.

An analytical descriptive procedure was used to show the overall characteristics of COVID-19 patients. Categorical data were formulated to obtain proportion. The normality test was conducted with the Kolmogorov-Smirnov test to find the data distribution. The numerical data would be shown as mean and standard of deviation if the distribution was normal or shown as median and range if the data distribution was not normal. The mean comparison test of serum IL-6 between the survivor and non-survivor group was analyzed using the Independent-T test for the normally distributed data, or the Mann-Whitney test for abnormally distributed data. Statistical analysis was performed using the SPSS 26th version (SPSS Inc. 26).

Results

The collected characteristics of the patients for this study were age, gender, and comorbidities as shown in Table 1. The normality test showed an abnormal distribution of the IL-6 concentration data, in contrast with normally distributed data of the age variable. As a result, the gender variable was examined using the Chi-square test, whereas the age variable was examined using the independent-T test.

The number of eligible subjects who met the inclusion criteria was 136, with a mean age of 56±13 years and a greater proportion of males (61,8%) than females (38,2%). The number of non-survivor subjects (36,4%) was lower than the number of survivors (65,4%). The data analysis revealed no statistically significant differences in age and gender between the survivor and non-survivor groups. Half of all subjects had hypertension, 32,4% had type 2 diabetes, and 27,9% had cardiovascular disease. There was no significant difference between the survivor and non-survivor groups in three comorbidities variables.

Table 1 Characteristics Baseline of the Subjects

Characteristics	Total	Outcomes		p Value
	n=136	Non-Survivor n=47	Survivor n=89	
Age (years)				
Mean ± SD	56 ± 13	57 ± 12	55 ± 14	0,542[a]
Gender				
Male	84 (61,8%)	31 (66,0%)	53 (59,6%)	0,465[b]
Female	52 (38,2%)	16 (34,0%)	36 (40,4%)	
Comorbidities				
Hypertension	68 (50%)	26 (56,5%)	42 (46,7%)	0,277[b]
Diabetes mellitus	44 (32,4%)	18 (39,1%)	26 (28,9%)	0,227[b]
Cardiovascular disease	38 (27,9%)	25 (27,8%)	13 (28,3%)	0,953[b]

Characteristics were reported in mean ± standard deviation and frequency (%). Analysis was performed using [a]Unpaired t-test [b]Chi-square test

Table 2 compares the IL-6 serum concentrations of survivors and non-survivors. The median and range of IL-6 concentrations in all included subjects were 16,5 pg/mL and 0,2 - 974,9 pg/mL, respectively. The median concentration of non-survivor group was significantly higher than the survivor group (41,4 pg/ml vs 10,8 pg/ml, p<0,001). Figure 1 shows the boxplot for the difference in IL-6 concentrations between the two groups.

Table 2 Serum IL-6 Concentration Difference Between Survivor and Non-survivor Group

Variable	Total n=136	Outcomes		p Value
		Non-Survivor n=47	Survivor n=89	
IL-6 (pg/mL)				
Median	16,5	41,4	10,8	<0,001*
Min - Max	0,2 – 974,9	2,0 – 974,9	0,2 – 180,0	

* p value is obtained from Mann-Whitney test between 2 groups

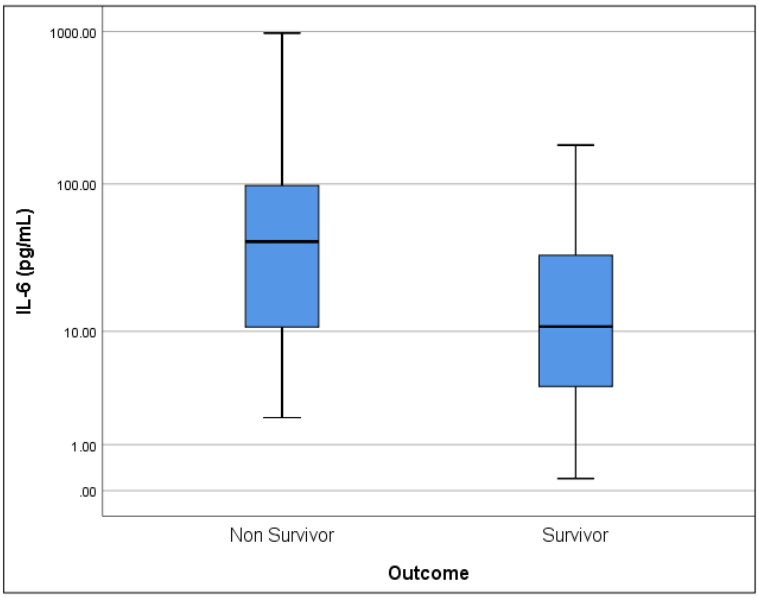

Fig. 1 The boxplot of IL-6 serum concentration difference between survivor and non-survivor group

Discussion

The baseline characteristics of the included patients revealed that the survivor group had a lower mean age (55±14 years) than the non-survivor group (57±12 years), although there was no statistically significant difference between the two groups (p=0.778). A study published in 2020 by Liu et al. found that elderly COVID-19 patients had a higher mortality rate than younger patients. The disease had a higher chance to progress into the severe stage due to the weakened immune system in the elderly population.[13] However, several studies described that negative outcomes can occur at any age, including children and adolescents.[7,14]

Gender data in the baseline characteristics showed a higher number of male patients than female patients, with no significant proportion difference between the survivor and non-survivor groups. A meta-analysis by Peckham et al. 2020 revealed no significant difference in the proportion of male and female COVID-19 patients. However, one study reported that males had a higher probability to require intensive care and a

higher chance of mortality in comparison to female patients.[15] Furthermore, Chen et al. 2020 stated that COVID-19 had a greater impact on elderly males with comorbidities.[3]

According to the findings of this study, 50% of the subjects had hypertension, 32,4% had diabetes, and 27,9% had cardiovascular disease. There was no significant difference between the survivor and non-survivor groups according to the comparison test. Yang et al. 2020 conducted a meta-analysis to assess the prevalence of comorbidities and their impact on COVID-19 patients. They discovered hypertension, respiratory disease, and cardiovascular disease as the risk factors for severe COVID-19.[16] Moreover, the mortality risk of COVID-19 was reported to be increased with the occurrence of cardiovascular disease, diabetes mellitus, and obesity as comorbidities.[7]

A statistically significant difference in IL-6 concentration was found between the two outcome groups ($p<0,001$). The median IL-6 level was found to be higher in the non-survivor group than in the survivor group, with values of 41,4 and 10,8 pg/mL, respectively. This difference was discovered for the median value and the test was conducted using a non-parametric test, hence the result was not as accurate as the parametric test. The severity of COVID-19 is linked to the immune response, which includes pro- and anti-inflammatory cytokines activity. Excessive pro-inflammatory cytokine secretion is associated with the severity of COVID-19 disease. It can cause acute respiratory distress syndrome as well as multi-organ failure, in which both are the causes of death in COVID-19 patients.[7]

This study found that the non-survivor group had significantly higher IL-6 concentrations than the survivor group. This finding was consistent with a study by Guirao et al. 2020, which found higher levels of IL-6 in patients with severe disease, patients on ventilation support, and deceased patients. The value of 35 pg/mL was determined to be the most effective cut-off value for predicting disease severity.[17] In addition, Gorham et al. 2020 investigated the IL-6 concentration in serial and discovered a significant difference in the maximum IL-6 concentration between the non-survivor and survivor groups.[18]

The higher IL-6 concentration in the non-survivor group reflects excessive and persistent IL-6 synthesis dysregulation. According to a study by Giamarellos-Bourboulis et al. 2020, patients with severe respiratory failure in COVID-19 experience immune dysregulation mediated by IL-6. This dysregulation is characterized by high pro-inflammatory cytokine production by monocytes and macrophages, as well as CD4+ lymphocyte depletion, both of which contribute to the progression of lung parenchymal inflammation. The direct role of IL-6 in COVID-19 pathogenesis was supported by evidence that IL-6 inhibition improves patient prognosis.[7,19] Wafa et al. 2021 compared several cytokines and inflammatory mediators and discovered that IL-6 was the most accurate inflammatory biomarker in predicting disease severity and patient death.[20] The significant difference in IL-6 levels between the survivor and non-survivor groups supported the hypothesis that an increase in inflammatory mediators is the underlying cause of CRS and is associated with a poor prognosis. A wide spectrum of immuno-active molecules, including interleukin (IL) and TNF-α, has been identified as potential contributors to CRS development. Among these interleukins, IL-6 as a multi-function inflammatory mediator has been recognized to play an important role in COVID-19-induced CRS interstitial pneumonia, and ARDS in severe COVID-19 patients.[21]

This study had several limitations. Firstly, there were gaps in the examination of additional comorbidities and the differentiation of COVID-19 diagnosis criteria. Additionally, there was little investigation of pharmacological use, particularly anti-IL-6 therapy. Further studies in a greater scope are required to obtain stronger data and evaluate the role of IL-6 level investigation as an early examination or serial for deciding therapy and prognosis of COVID-19.

This study reveals that the non-survivor group of hospitalized COVID-19 patients had considerably higher serum concentrations of IL-6 than the survivors group. There were no discernible differences in the two groups' ages or genders. This finding may serve as the initial support for using IL-6 as a laboratory marker to

assess the severity and mortality rate of COVID-19. Additionally, this study may serve as a starting point for future investigations into the significance of IL-6 as a COVID-19 mortality predictor in Indonesia, particularly in West Java, using more extensive comorbidity data and sample sizes. Furthermore, it is possible to explore the utility of anti-IL-6 therapy further as one of the current recommended therapies for COVID-19.

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