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ORIGINAL RESEARCH

Interleukin-1β and Interleukin-10 Profiles and Ratio in Serum of COVID-19 Patients and Correlation with COVID-19 Severity: A Time Series Study

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Background: Coronavirus disease-19 (COVID-19) can, in severe cases, lead to cytokine-release syndrome owing to an excessive immune response. The release of different cytokines aggravates disease severity. IL-1β is a pro-inflammatory cytokine, while IL-10 is an anti-inflammatory cytokine, and both are involved in the human immune response to infection. This study aimed to determine whether serum levels of IL-1β and IL-10 and the ratio of the two over time in patients with COVID-19 could facilitate early identification of disease severity.

Methods: An analytical, observational time-series design was employed. Fifty participants were enrolled between May and October 2020 and were divided into two groups—non-severe (n = 20), and severe (n = 30). IL-1β and IL-10 were analyzed using BD cytometric bead array sets. Association of the IL-1β:IL-10 ratio with COVID-19 severity was analyzed using a Mann–Whitney test and Fisher's exact test. Optimal cut-off values to predict disease severity were determined by Youden's index.

Results: In non-severe and severe groups, the median serum levels of IL-1β decreased on day 3 (1.72 ng/mL and 2.10 ng/mL, respectively), then increased on day 6 (2.05 ng/mL and 3.31 ng/mL, respectively). However, the median of IL-10 increased on day 3 (1.88 ng/mL and 2.30 ng/mL, respectively) and day 6 (2.02 ng/mL and 2.39 ng/mL, respectively). There was no significant association between the IL-1β:IL-10 ratio and COVID-19 severity at any time-point ($p>0.05$). The cutoff value of serum IL-10 between the two groups on days 0, 3, and 6 was 1.09 pg/mL (sensitivity: 66.6%; PPV: 71.4%), 2.11 pg/mL (sensitivity: 67.7%; PPV: 50.0%), and 2.08 pg/mL (sensitivity: 78.6%; PPV: 70.9%), respectively.

Conclusion: The IL-1β:IL-10 ratio was not correlated to COVID-19 severity. However, owing to its high sensitivity, IL-10 may be a potential biomarker for disease severity in severe COVID-19.

Keywords: interleukin, IL-1β, IL-10, severity, COVID-19

Introduction

Coronavirus 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported at the end of 2019 and rapidly became a global pandemic. Although COVID-19 is associated with several systemic symptoms, the dominant symptom is respiratory inconvenience. Human-to-human transmission is the confirmed etiology of this disease.[1–3] Mortality in patients with COVID-19 is predominantly due to respiratory failure arising from severe pneumonia.[4] COVID-19 pneumonia generally causes acute respiratory distress syndrome (ARDS), which is the most common reason for mortality.[5] The occurrence of ARDS in patients with COVID-19 is predicted to be caused by an excessive immune response that culminates in cytokine-release syndrome (CRS), commonly known as "cytokine storm." Cytokine storm, as the consequence of ARDS, results from the combined effects of many immune-active molecules including interferons, interleukins, chemokines, colony-stimulating factors, and TNF-alpha.[6] SARS-CoV-2 enters lung cells by binding to the ACE-2 receptor and undergoes intracellular viral replication. Cell stress then occurs, stimulating

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pyroptosis, which produces several damage-associated-molecular patterns. Consequently, resident lung cells begin to secrete chemokines (CXCL10, CXCL8, CXCL9, CCL2, CCL3, CCL5) and this is accompanied by the recruitment of immune cells expressing the specific chemokine receptors. Through a multistep process involving adherence and migration across the endothelium, followed by trafficking through the interstitium, the immune cells reach the site of the infection. Infiltrating immune cells secrete pro-inflammatory cytokines (IFNα, IFNγ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNFα, and TGFβ) which the onset and the maintenance feed-back loop will cause the maintenance of chemokines secretion.[7] CXCL10 is a chemokine that is able to recruit more monocytes, macrophages, and neutrophils to the infection site, resulting in the production of more pro-inflammatory cytokines at the site, culminating in the occurrence of a cytokine storm. This condition causes damage to the alveolar epithelium and the epithelium-endothelium barrier, and consequently ARDS occurs, which is identified by tissue hypoxia and hypoxemia.[7,8]

In cytokine storm, the levels of several cytokines such as IL-1β, IL-10, and TNF-α are increased, which correlate with disease severity. Interleukin-1β (IL-1β) is a member of the interleukin 1 family of cytokines that are instrumental in inflammatory responses. IL-1β is an important, robust pro-inflammatory cytokine involved in the body's immune response against infection and injury. The index between the effective and toxic functions of IL-1 is understood to be very narrow, therefore regulation of IL-1 in the human body is tightly controlled.[9,10] Furthermore, IL-1β is a gatekeeper of inflammation owing to its ability to regulate several cascades through other inflammation-associated molecules, such as interleukin-6 (IL-6). IL-6 regulates the acute phase response and is involved in acute inflammation, which ultimately leads to tissue damage.[6,11] Interleukin 10 (IL-10) is a cytokine with a powerful anti-inflammatory function, and plays a key role in restricting the host's immune response against pathogens, thereby avoiding damage to the host and maintaining normal tissue homeostasis. IL-10 has strong anti-inflammatory and immunosuppressive activity on myeloid cell function, which forms the basis of its involvement in acute and chronic inflammatory diseases. IL-10 is identified as an inhibitory factor of Th1 secreted by Th2 cells.[12] There is increasing research on IL-10 as a potential biomarker for COVID-19 severity, and high expression of IL-10 may predict a worse prognosis in patients with COVID-19.[13]

The simultaneous increase in the concentrations of pro-inflammatory and anti-inflammatory cytokines is evidenced by two cytokines, IL-1β and IL-10. Both cytokines were reportedly increased in patients with severe COVID-19; however, there are currently no studies comparing the effect and influence of both cytokines together. According to the established facts and theories, both cytokines are known to potently cause tissue damage. We believe that through the comparison of both IL-1β and IL-10, it may be possible to detect early aggravation of COVID-19 severity, which will facilitate appropriate and individualized management strategies in patients with this disease. Therefore, this study focuses on the profile of IL-1β and IL-10, as well as the ratio of the two cytokines, in the severity of COVID-19 over time.

Materials and Methods

Study Design and Subjects

This study has an analytical, observational time-series design. All study participants had COVID-19 and were admitted to the Dr Soetomo General Hospital, Surabaya, Indonesia from May to October 2020. The study samples were obtained from patients who met the inclusion and exclusion criteria. Consecutive sampling method was used. The inclusion criteria were male or female patients diagnosed with COVID-19, aged ≥18 years and willing to participate in the research for which they voluntarily provided consent. The exclusion criteria were patients who did not survive or were discharged from the hospital before samples were obtained on day 0, 3, and 6, or patients with other autoimmune diseases.

Definition of Variables

COVID-19 was diagnosed in patients hospitalized in the isolation ward of Dr Soetomo General Hospital. The diagnosis was confirmed by real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay for nasal and pharyngeal swab specimens. IL-1β and IL-10 were analyzed on days 0, 3, and 6 using a BD FACSCalibur (BD Biosciences, USA) with pg/mL units with the analysis conducted in the Clinical Pathology Laboratory of Dr Soetomo General Hospital. COVID-19 severity was defined as the degree of severity of COVID-19 disease according to the WHO 2021 categorization:[14] *Mild*, including symptomatic patients who meet the COVID-19 case definition without evidence of

viral pneumonia or hypoxia; *Moderate* (pneumonia), patients who have clinical symptoms of pneumonia such as fever, cough, rapid-breathing, and/or dyspnea with no sign of severe pneumonia and SpO2 ≥90% in room air; *Severe* (severe pneumonia), patients with clinical sign of pneumonia (fever, cough, rapid-breathing, and/or dyspnea) plus one of the following signs: respiration frequency >30x/min, severe respiratory distress, and/or SpO2 <90% in room air; *Critical*, compromises of patients with ARDS, sepsis, and septic shock. In this study, the severity was divided into two groups based on WHO guidelines:[14] non-severe (mild – moderate) and severe (severe – critical) groups. Day 0, day 3, and day 6 are the time points of the patients hospitalized at admission, the third day, and the sixth day in the isolation ward of Dr Soetomo General Hospital. Autoimmune disease is a clinically diagnosed condition, such as arthritis gout, osteoarthritis, multiple myeloma, systemic lupus erythematosus, and rheumatoid arthritis obtained from anamnesis, or physical and laboratory examinations.

IL-1β and IL-10 Analysis

Blood samples were collected from the patients on days 0, 3, and 6 using a 5-cc spuit and were transferred to a serum separator tube. Samples were placed in a cool box and transported to the Clinical Pathology Laboratory of Dr Soetomo General Hospital for centrifugation and storage at −80°C. IL-1β and IL-10 serum levels were measured in the Clinical Pathology Laboratory using BD Cytometric Beads Array (CBA) Human IL-1β and IL-10 Flex sets and a BD FACSCalibur flow cytometer. Briefly, microbead populations with distinct fluorescent intensities were pre-coated with capture antibodies specific for each cytokine. Fifty microliters of Flex set standard dilution or serum samples were added to the appropriate assay tubes (BD). Next, 50 µL mixed capture beads that consist of antibodies specific for the cytokines was added and the samples were incubated for 1 h in the dark at room temperature. Subsequently, 50 µL mixed PE detection reagent was added and incubated for 2 h in the dark at room temperature. The samples were then washed and centrifuged at 200 *g* for 5 min and the pellet was resuspended in 300 µL wash buffer. Samples were analyzed using a calibrated FACSCalibur flow cytometer. Data were computed using the standard reference curve and FCAP Array software.

Ethics Statement

Written informed consent for publication was obtained from the study participants and/or relatives of the participant. The study was performed following the ethical principles of the Declaration of Helsinki and was approved by the institutional review board of the Dr Soetomo General Hospital (registration number: 1953/KEPK/IV/2020).

Data Analysis and Statistics

Categorical variables were described as frequency rates and percentage, and continuous variables were described using mean±standard deviation (SD) and median (IQR). A normality test was performed using Kolmogorov–Smirnov test. Normally distributed data were analyzed using an independent *t*-test, while non-normally distributed data were analyzed using the Mann–Whitney *U*-test. Non-normally distributed data from repeated measures were also compared using the generalized linear mixed model. The area under the curve (AUC) and 95% confidence interval (CI) of the receiver operating characteristic (ROC) curve were used to assess the accuracy of each biomarker in predicting COVID-19 severity. The severity of COVID-19 was predicted by optimal cut-off points determined by Youden's index. All data and statistical analysis were conducted using IBM SPSS Statistics software version 21.0 (IBM Corp., Armonk, NY, USA). Two-sided *p*-values <0.05 were considered to indicate statistically significant differences.

Results

Patient Characteristics

The subjects of this study (n = 50) were those with COVID-19 and hospitalized in the isolation ward of Dr Soetomo General Hospital, Surabaya, Indonesia from May to October 2020. Patient characteristics were analyzed based on the disease severity. There were no differences in age, gender, and comorbidities between the non-severe and severe groups

of patients ($p>0.05$). Symptom characteristics such as shortness of breath were significantly different between the two groups ($p<0.001$) (Table 1).

Laboratory examination results as well as disease severity were analyzed using *t*-tests and Mann–-Whitney tests. Laboratory analysis included tests for leucocytes, lymphocytes, neutrophils, procalcitonin, CRP, ferritin, and D-dimer, which were all significantly different between the non-severe and severe groups of patients ($p<0.05$) (Table 1).

COVID-19 Severity

Subject classifications were based on COVID-19 severity. The study involved 50 participants of whom 7 (14%) had critical disease; 23 (46%) had severe disease; 19 (38%) had moderate disease; and 1 (2%) had mild disease at admission to the hospital. The severity changed on day 3 and day 6 according to clinical conditions of the patients. Patients who initially had mild disease showed an aggravation on severity on day 3 (2 subjects [4%]) and day 6 (4 subjects [8%]). On day 3, the proportion of subjects with moderate disease was decreased (17 subjects [34%]) but increased again on day 6 (18 subjects [36%]), while in the severe disease group, the proportion of subjects was increased on day 3 (29 subjects [58%]) but decreased on day 6 (26 subjects [52%]). Finally, the proportion of subjects in the critical disease group was decreased on day 3 (2 subjects [4%]) and remained constant at day 6.

Participants were divided into two large groups—the non-severe group (n = 30 [60%]) and the severe group (n = 20 [40%]). The non-severe group contained patients with moderate and mild disease, while the severe group comprised

Table 1 Characteristics and Severity of COVID-19 of the Subject

Characteristics	Non-Severe	Severe	p-value
Age (Median (min-max))	50 (26–61)	52 (25–70)	0.218
Gender			0.451
Male	9 (45%)	18 (60%)	
Female	11 (55%)	12 (40%)	
Early symptoms			
Shortness of breath	8 (40%)	29 (96.7%)	<0.001
Fever	13 (65%)	18 (60%)	0.953
Cough	13 (65%)	15 (50%)	0.450
Others	5 (25%)	3 (10%)	0.240
Comorbid			
Diabetes mellitus	7 (35%)	13 (43%)	0.560
Hypertension	3 (15%)	6 (20%)	0.655
Obesity	3 (15%)	8 (26.7%)	0.334
Stroke	1 (5%)	1 (3.3%)	0.080
Chronical kidney disease	0	2 (6.6%)	0.771
Asthma	1 (5%)	2 (6.6%)	0.336
Tuberculosis	1 (5%)	3 (10%)	0.557
Laboratory (Median (min - max))			
Leucocytes (10^3/μL)	7620 (6650–15,820)	9250 (7380–18,420)	0.022
Lymphocytes (10^3/μL)	1.21 (0.51–2.40)	1.16 (0.47–3.88)	0.552
Lymphocytes %	14.75 (4.50–32.30)	10.4 (3.80–36.30)	0.025
Neutrophils (10^3/μL)	5.33 (2.71–14.34)	8.59 (3.39–8.59)	0.007
Neutrophils %	76.85 (54.5–90.60)	82.15 (55.80–92.80)	0.027
Procalcitonin (ng/mL)	0.09 (0.01–0.47)	0.29 (0.06–6.68)	0.001
CRP (mg/dL)	4.60 (0.10–27.10)	5.80 (0.20–23.30)	0.435
Ferritin (μg/L)	494 (26–1878)	1233 (42–6498)	0.005
Fibrinogen (mg/dL)	467 (148–755)	419 (184–851)	0.748
D-dimer (ng/mL)	850 (190–7410)	1910 (330–35,200)	0.025

Table 2 IL-1β Serum Levels and Its Correlation with COVID-19 Patient's Severity

IL-1β	Non-Severe				Severe				Non-Severe and Severe
Time-Points	Min	Max	Median	*P-value	Min	Max	Median	**P-value	***P-value
Day 0	1.23	8.17	2.92	0.035	1.87	96.92	2.94	0.088	0.513
Day 3	0.98	214.19	1.72		0.90	121.63	2.10		0.646
Day 6	1.53	47.02	2.05		0.61	18.01	3.31		0.113

Notes: *P-value of the IL-1β serum levels differences on day 0, 3, and 6 in non-severe group. **P-value of the IL-1β serum levels differences on day 0, 3, and 6 in severe group. ***P-value of the IL-1β serum levels comparison between non-severe and severe groups on day 0, 3, and, 6.

patients with severe and critical disease. The distribution of this severity then changed on day 3 and day 6 according to the subjects' clinical course. On day 3, the severity composition was changed by one subject experiencing a worsening condition (non-severe: 19 [38%] and severe: 31 [62%]), while on day 6, an improvement occurred in three subjects (non-severe: 22 [44%] and severe: 28 [56%]).

IL-1β and IL-10 Serum Levels in COVID-19 Patients on Days 0, 3, and 6

The correlation of IL-1β serum levels and COVID-19 severity was also analyzed. The serum levels of IL-1β between non-severe and severe groups at each time point were compared using the Mann–Whitney *U*-test (Table 2). The range of IL-1β was quite wide and volatile on day 0, day 3, and day 6. IL-1β serum levels in the non-severe group on days 0, 3, and 6 showed significant differences, but not in the severe group. However, IL-1β serum levels showed no significant differences with respect to disease severity. The median value of IL-1β on day 3 was decreased but increased again on day 6. On day 3, IL-1β serum levels showed a tighter minimum and maximum value range. The median value of the non-severe group was always lower compared with that of the severe group on days 0, 3, and 6 (Figure 1).

ROC curve analysis was also performed (Table 3). On day 0, the IL-1β serum level was ≥2.77 pg/mL, which may differentiate the severity with a sensitivity of 60.0%, consistent with the positive prediction value of 64.2%. On day 3, the serum level was ≥1.68 pg/mL and on day 6 it was ≥2.12 pg/mL, which showed progression of severity despite the sensitivity and specificity being low at both time points.

The serum levels of IL-10 were also analyzed for correlation with COVID-19 severity and were examined at each time point between the non-severe and severe groups using the Mann–Whitney *U*-test. The differences between IL-10 serum levels in the non-severe and severe groups on days 0, 3, and 6 were statistically significant (Table 4). The median value of IL-10 serum levels on days 3 and 6 kept increasing in both the non-severe group and the severe group. The range of minimum and maximum values of IL-10 in the non-severe group was decreased on days 3 and 6, and was also decreased in the severe group at these time points (Figure 1). The median value of the non-severe group was always lower compared with that of the severe group at each time point. There were no significant differences in IL-10 serum levels on days 0, 3, and 6 with respect to disease severity.

ROC curve analysis showed that the IL-10 serum level at admission was ≥1.09 pg/mL, which may differentiate non-severe and severe groups with a sensitivity of 66.6%, consistent with the positive prediction value of 71.4% (Table 3). On

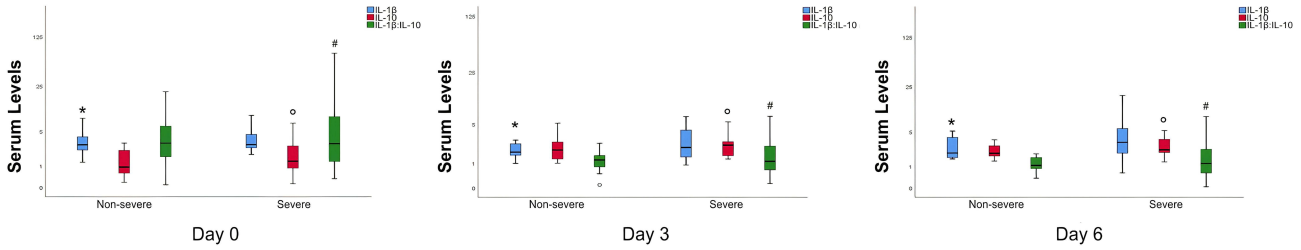

Figure 1 Serum levels and correlations with COVID-19 patient's severity. Serum levels of IL-1β (blue), IL-10 (red), and the ratio of IL-1β:IL-10 (green) in group of patients diagnosed with severe (right) and non-severe (left) COVID-19 on days 0, 3, and 6. IL-1β levels, in non-severe group, d0 x d3 x d6 (p= 0.035); IL-10 levels, in severe group, d0 x d3 x d6 (p= 0.001); IL-1β:IL-10 levels, in severe group, d0 x d3 x d6 (p= 0.002). *Significant correlation of IL-1β; °Significant correlation of IL-10; #Significant correlation of IL-1β:IL-10.

Table 3 IL-1β, IL-10, and IL-1β/IL-10 Value as Severity Biomarker

Time-Points	Interleukin	Cut Off	Non-Severe	Severe		CI 95%
Day 0	IL-1β	≥ 2.77	10	18	Sen = 60.0	40.6–77.3
					Spec = 50.0	27.2–72.8
		< 2.77	10	12	PPV = 64.2	51.5–75.3
					NPV = 30.9	30.9–60.7
	IL-10	≥ 1.09	8	20	Sen = 66.6	47.1–82.7
		< 1.09	12	10	Spec = 60.0	36.0–80.8
					PPV = 71.4	58.0–81.9
					NPV = 54.55	39.2–69.0
	IL-1β/IL-10	≥ 3.21	10	18	Sen = 50.0	31.3–68.7
		< 3.21	10	12	Spec = 55.0	31.5–76.9
					PPV = 62.5	47.7–75.2
					NPV = 42.3	30.0–55.5
Day 3	IL-1β	≥ 1.68	9	19	Sen = 61.3	42.1–78.5
		< 1.68	10	12	Spec = 50.0	24.4–71.4
					PPV = 62.9	53.2–75.9
					NPV = 43.4	28.1–58.4
	IL-10	≥ 2.11	9	21	Sen = 67.7	48.6–83.3
		< 2.11	10	10	Spec = 52.3	28.8–75.5
					PPV = 70.0	57.8–79.9
					NPV = 50.0	33.9–66.0
	IL-1β/IL-10	≥ 1.07	11	17	Sen = 54.8	36.0–72.6
		< 1.07	8	14	Spec = 42.1	20.2–66.5
					PPV = 60.7	48.4–71.8
					NPV = 36.3	36.3–52.3
Day 6	IL-1β	≥ 2.12	9	20	Sen = 60.0	51.3–86.7
		< 2.12	13	8	Spec = 55.0	34.4–76.8
					PPV = 66.6	54.2–77.1
					NPV = 47.8	44.9–76.3
	IL-10	≥ 2.08	9	22	Sen = 78.6	59.0–91.7
		< 2.08	13	6	Spec = 59.1	36.3–79.2
					PPV = 70.9	58.8–80.7
					NPV = 68.4	49.5–82.6
	IL-1β/IL-10	≥ 1.14	9	16	Sen = 57.1	37.1–75.5
					Spec = 59.1	36.3–79.2
		< 1.14	13	12	PPV = 64.0	49.4–76.3
					NPV = 52.0	38.9–65.2

Abbreviations: Sen, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Table 4 IL-10 Serum Levels and Its Correlation with COVID-19 Patients Severity

IL-10	Non-Severe				Severe				Severe and Non-Severe
Time-Points	**Min**	**Max**	**Median**	***P-value**	**Min**	**Max**	**Median**	****P-value**	*****P-value**
Day 0	0.17	27.45	0.90	0.047	0.12	6.85	1.30	<0.001	0.417
Day 3	0.99	18.98	1.88		1.24	20.29	2.30		0.255
Day 6	1.36	6.51	2.02		1.29	58.87	2.39		0.065

Notes: *P-value of the IL-10 serum levels differences on day 0, 3, and 6 in non-severe group. **P-value of the IL-10 serum levels differences on day 0, 3, and 6 in severe group. ***P-value of the IL-10 serum levels comparison between non-severe and severe groups on day 0, 3, and, 6.

Table 5 The Ratio of IL-1β and IL-10 Serum Levels on Day 0, 3, and 6

IL-1β/IL-10	n	Mean ± SD	Median (Min – Maks)
Day 0	50	6.62 ± 11,620	3.12 (0.08–73.51)
Day 3	50	3.84 ± 12,198	1.12 (0.08–64.58)
Day 6	50	2.49 ± 3525	1.13 (0.03–19.05)

Table 6 The Ratio of IL-1β and IL-10 Serum Levels and Its Correlation with COVID-19 Patients Severity

IL-1β/IL-10	Non-Severe				Severe				Severe and Non-Severe
Time-Point	Min	Max	Median	*P-value	Min	Max	Median	**P-value	***P-value
Day 0	0.08	20.54	3.12	0.086	0.31	73.51	3.07	0.002	0.751
Day 3	0.08	64.58	1.18		0.13	60.82	1.10		0.865
Day 6	0.36	19.05	1.05		0.03	8.86	1.18		0.891

Notes: *P-value of the IL-1β/IL-10 serum levels differences on day 0, 3, and 6 in non-severe group. **P-value of the IL-1β/IL-10 serum levels differences on day 0, 3, and 6 in severe group. ***P-value of the IL-1β/IL-10 serum levels comparison between non-severe and severe groups on day 0, 3, and, 6.

days 3 and 6, the serum IL-10 level was ≥2.11 pg/mL and ≥2.08 pg/mL, respectively, which showed persistence of severity that could likely facilitate clinical decisions regarding appropriate therapy for severe and mild cases of pneumonia, with a sensitivity of 67.7% and 78.6%, respectively.

Ratio of Serum IL-1β and IL-10 Levels on Days 0, 3, and 6 and Correlation with COVID-19 Severity

Changes in the ratio of serum IL-1β and IL-10 levels were analyzed when patients were hospitalized on day 0, day 3, and day 6. The mean and median values decreased on day 3 and day 6. The range of minimum to maximum values was also narrower than on day 0 (Table 5). ROC curve analysis of the ratio of IL-1β and IL-10 was also performed on days 0, 3, and 6 in both the non-severe and severe groups. On day 0, the ratio of IL-1β and IL-10 was ≥3.21 pg/mL, which could have differentiated between severe and non-severe disease but the sensitivity was only 50.0%—consistent with the positive prediction value of 62.5% (Table 3). On days 3 and 6, the ratio of serum IL-1β and IL-10 levels was ≥1.07 pg/mL and ≥1.14 pg/mL, respectively, which showed progression of severity, however, both time points had low sensitivity.

The normality test showed that the ratio of IL-1β and IL-10 in the severe and non-severe groups on days 0, 3, and 6 did not present a normal distribution ($p<0.05$); hence the difference of the IL-1β and IL-10 ratio between the two groups was analyzed using the Mann–Whitney *U*-test. No significant difference was found on days 0, 3, and 6 in either group ($p>0.05$) (Table 6). The median values of the IL-1β/IL-10 ratio on days 3 and 6 were both decreased in the severe and non-severe groups. The range of minimum and maximum values was tighter in both groups on day 6. The median of the non-severe group was higher compared with that of the severe group on days 0 and 3 but was lower on day 6 (Figure 1). Statistical analysis showed that there was no significant difference in the IL-1β/IL-10 ratio between the non-severe and severe groups on days 0, 3, and 6 ($p>0.05$). This also means that there was no correlation between the IL-1β/IL-10 ratio and COVID-19 severity in the patients on days 0, 3, and 6. However, on day 6, the ratio of IL-1β and IL-10 serum levels in the non-severe group was lower compared with that in the severe group ($p>0.05$) (Table 6).

Discussion

This study demonstrated that the proportion of COVID-19 patients with severe infection (ie severe and critical classification per WHO) was greater (60%) than those with mild and moderate infection (non-severe) (40%). This research was conducted in Dr Soetomo General Hospital, Surabaya, Indonesia, which is a referral hospital for eastern Indonesia and typically most admissions are patients in severe conditions. Our results suggested that the most frequent symptom in the study participants was shortness of breath (74%). This finding was supported by He et al[15] who reported

that patients with early symptoms of shortness of breath, hemoptysis, anorexia, diarrhea, stomach ache, or fatigue required more clinical attention as these symptoms tend to be indicative of more severe disease and/or potentially death, thus warranting stricter observations. Studies by Suryananda et al[16] and Saputra et al[17] also found that more than 70% patients had shortness of breath as the symptom.

Our study suggested that leucocyte count had a correlation with the severity of COVID-19. Henry et al[18] stated that patients with severe disease only had a minor increase in leucocyte counts, while patients who did not survive had significantly high counts. A previous study[19,20] suggested that patients with severe illness are more likely to have a lower lymphocyte count, but a higher count of leucocytes and a higher neutrophils-lymphocytes (NLR) ratio. The decrease in lymphocytes but high serum ferritin levels was reported as a prognostic predictor in COVID-19 patients.

The current study found a decrease in lymphocytes and an increase in neutrophils and serum ferritin levels, which correlated with disease severity. Our study also showed that procalcitonin was correlated with severe disease. A previous study mentioned that high procalcitonin levels may be affected by secondary infection stage.[18] D-dimer was found to be related to disease severity in the present study (median value: 1910 ng/mL), and this finding was supported by Ibanez et al[21] who reported that D-dimer levels of >1000 ng/mL facilitated early identification of a worse prognosis in patients with COVID-19.

We found that the median IL-1β concentration in the non-severe group tended to decrease on days 3 and 6 compared with that on day 0, which may be therapeutic according to some previous studies. In the severe group, the median concentration of IL-1β was increased on day 6 even though it had decreased on day 3, indicating that the inflammatory process would likely continue to increase. A study by Bell et al[22] suggested that IL-1β activity was a host response at the infection site of SARS-CoV-2 and was more likely induced by increased viral replication. IL-1 actively participates in the inflammation response against infection, and the main resources are activated monocytes and macrophages. SARS-CoV -2 seems to work on activation and maturation of IL-1β that will activate other pro-inflammatory cytokines such as IL-6 and TNF-α. IL-1β is a component of the cytokine storm resulting from coronavirus infection. The elevation of antagonist receptor levels, IL-1 (IL-1RA), in severe COVID-19 cases has been correlated with increased viral load, loss of lung function, lung injury, and death risk.[23]

IL-1β has widely known to facilitate enhanced alveolar permeability in animals and humans. An experimental study on lung injury induced by IL-1β proved that the injury could be reduced by administration of IL-1RA. IL-1RA is a natural restrictor of the pro-inflammatory cytokine IL-1β, which is a very active cytokine found in the lungs of patients with non-COVID-19 ARDS. The decrease of IL-1RA in bronchoalveolar lavage (BAL) samples is correlated with higher mortality of ARDS. A study by Balnis et al[24] showed that the elevation of IL-1RA indicates respiratory failure induced by COVID-19 ARDS, which is different from non-COVID-19 ARDS, and supported the potential pharmacological modulation of IL-1RA recently suggested for patients with COVID-19. A higher plasma level of IL-1RA was positively correlated with mortality in a group of patients with critical COVID-19, even though the IL-1β level did not show significant correlation with mortality.[24] The role of IL-1β in disease severity has been described in the above studies. In the present study, the serum level of IL-1β was higher in the severe group compared with that in the non-severe group on days 0, 3, and 6, despite the insignificant differences with respect to severity of disease.

Gabay et al[25] found that IL-1β was increased in severe patients compared with moderate patients at most of the analyzed time points in their study. The IL-1 antagonist receptor (IL-1RA), induced by IL-1R signaling as a negative feedback regulator exhibited elevated levels in ICU patients from day 10 of disease onset.[25] Bell et al[22] presented variation in the bioactivity of IL-1β during the hospitalization period, and higher expression was seen in patients at admission. Li et al[26] analyzed the correlation between cytokine levels and explored the effects of longitudinal cytokine changes in severe patients in the ICU for 14 days. IL-1β was increased from day 4 and decreased on day 14 in the non-surviving group, while in the surviving group, IL-1β tended to decrease on the first day until day 13 and then increased on day 14.[26]

The current study showed statistically significant differences of IL-10 serum levels in the non-severe and severe groups on days 0, 3, and 6. The median concentration of IL-10 on days 3 and 6 was increased in both the non-severe and severe groups compared to day 0. In the non-severe group, the median of IL-10 was always lower than that of the severe group on days 0, 3, and 6. However, there were no significant differences in IL-10 correlation with severe disease on days

0, 3, and 6. IL-10 may be affected by various factors such as SARS-CoV-2 infection itself or another reason that was not analyzed in this study. IL-10 is a multifunctional, multi-source, and multi-regulatory cytokine. The fact that other cytokines present in the cellular environment affect the production rate of IL-10 justifies this complexity. An important example from this process is the TH1 interferon-gamma (IFN-γ) cytokine that blocks the production of IL-10 from macrophage and dendritic cells. IL-10 has a dominant function as an immunosuppressive cytokine.[27] Different immunological backgrounds allow detrimental activity of IL-10 to the host. This occurs when immune response is not exaggerated but is rather protective towards the host. Suppression of the immune response by an abundance of IL-10 is deemed to be inappropriate, as the effect is a failure of adequate infection clearance, leading to chronic infection. Immune regulation by IL-10 is thus a double-edged sword, acting in the interests of the host, not in all but in some situations.[27]

Several authors have detected these interleukins in patients with COVID-19 and correlated the levels with disease severity and progression. IL-10 may be overexpressed in response to SARS-CoV-2. IL-10 was found to be higher in patients with advanced age owing to a hyper-inflammatory response and may be related to the reduction of T-cell receptors in the elderly. As in the case of other cytokines, IL-10 levels were higher in COVID-19 patients compared with patients with SARS-CoV infection or MERS.[23] Another study by Han et al[28] also used IL-10 as a predictor to identify patients with moderate-to-severe COVID-19 at higher risk of worsening condition. Our study found different results to previous studies as IL-10 was not significantly correlated with COVID-19 severity at any time points; however, the IL-10 serum concentration in the non-severe group in our study was always lower than that of the severe group at days 0, 3, and 6. Decreased levels of circulating IL-10 in patients with severe COVID-19 may indicate that the immune regulatory network is activated to restrict the magnitude of the immune response and repair damage.[26] ROC curve analysis showed that on day 0, the IL-10 level was ≥1.09 pg/mL which may distinguish non-severe and severe cases with a sensitivity of 66.6%. However, on days 3 and 6, IL-10 showed a value of ≥2.11 pg/mL and 2.19 pg/mL with a sensitivity of 60.0% and 73.3%, respectively. Han et al[28] conducted ROC analysis of IL-10 values with respect to COVID-19 severity and found that the IL-10 value was above the ROC curve and was interpreted as a fairly good marker for assessing disease severity.

In our study, although the IL-1β/IL-10 ratio decreased on days 3 and 6, the change was not statistically significant. The range of minimum and maximum values was also narrower on days 3 and 6 than on day 0. Studies on longitudinal cytokine profiles in patients across all severities of COVID-19 are still limited.[29,30] To our knowledge, the correlation between cytokines and specific clinical points such as severity of organ dysfunction has not yet been explored. This is unfortunate because it is an important reference for appropriate timing of immunotherapy in optimizing efficacy.[31]

The early stage of infection, which is <5 days of symptoms, leads to a respiratory phase that is more prominent in those with early signs of cytokine storm syndrome, so the inflection point of the disease usually occurs between days 5 and 7. This interval is the time point for targeted immunomodulatory therapy as it may be most beneficial in reducing mortality. Multiorgan dysfunction syndrome (MODS) is predicted to start from day 10.[31] A study by Ling et al[32] performed an assessment of the cytokine profile of blood samples collected during the early (<7 days) and late phase (8–12 days) of symptoms onset. The level of IL-1β in the initial phase was not significantly different in patients with mild, moderate, or severe symptoms. However, in the late phase, the IL-1β level began to increase in all disease severities ($p>0.05$). The study also demonstrated that IL-10 values in the early phase presented significant differences, particularly in patients with moderate and severe disease. Furthermore, IL-10 values in the late phase showed insignificant changes in mild and moderate severity, but sharply escalated in severe or critical disease. Overall, these observations suggested that worsening of the patient's condition usually occurred around days 8–12 from the onset of symptoms and was not affected by uncontrolled viral load.

Lucas et al[29] described longitudinal cytokine correlations measured in days from symptom onset (DfSO) and revealed that large differences in immune phenotype between moderate and severe disease were evident after day 10 of infection. Patients with severe or moderate disease showed similar intensity and correlation markers in the first 10 DfSO. After day 10, this marker continued to decline in patients with moderate disease. In contrast, patients with severe COVID-19 maintained higher cytokine levels. Primarily, additional correlations between cytokines appeared in patients with severe disease after day 10.

In the current study, there was no correlation between the ratio of IL-1β and IL-10 between non-severe and severe groups on days 0, 3, and 6 ($p>0.05$). On days 0 and 3, the IL-1β/IL-10 ratio for non-severe patients was always higher

than that for severe patients, but on day 6, these results were reversed. Li et al[26] found that IL-10 was a predictor of survival in ICU patients regardless of other severity factors. The serum level of IL-10 was increased, and these escalations were correlated with elevated levels of pro-inflammatory factors despite IL-10 usually being considered as an anti-inflammatory molecule. Some other studies[26,33] have suggested that anti-inflammatory and pro-inflammatory reactions coexist in patients with sepsis. IL-10 enables escalation of IL-1RA production, which may be the reason for increased IL-1RA levels in parallel with IL-10 levels and concomitant with enhancement of disease severity.[32] However, IL-1a and IL-1b were not consistently elevated in severe disease.[34]

This study has some limitations, including the need for a future study that measures the correlation of IL-1β and IL-10 profile and the ratio with COVID-19 severity. In addition, the study had a larger population of subjects in the non-severe group compared with the severe group. Our observations were also only conducted over 6 days; hence, this may be the reason no correlation was found between the ratio of IL-1β and IL-10 levels between the non-severe and severe groups at all assayed times. We did detect a trend of IL-1β elevations in the non-severe group, which may have shown therapeutic value if the study had conducted over a longer period. Furthermore, the clinical course could be observed in more detail if analyses were performed at the time of symptoms onset; in this study, it was only at admission.

Conclusion

The different results in our study compared with those of several previous studies on the correlation of IL-1β and IL-10 serum levels with COVID-19 severity may be because of the differences in disease time-points analyzed and the balance of IL-1β and IL-10 serum levels. The current study found no correlation between the IL-1β and IL-10 ratios with COVID-19 severity; however, our findings showed that on day 6 of observation, IL-1β and IL-10 levels were elevated in the severe group, while in the non-severe group, the decrease of IL-1β was offset by IL-10 elevation. The IL-1β/IL-10 ratio in the severe group was decreased on day 3 and day 6 compared with day 0. The ratio of IL-1β and IL-10 serum levels in the severe group at the early time-points was lower compared with that in the non-severe group, but higher on day 6. ROC curve analysis showed that IL-10 with a cutoff point of 2.19 pg/mL on day 6 in the severe group may be an effective marker to assess COVID-19 severity with a sensitivity of 73.3%. These findings proved that in severe COVID-19, the inflammation escalated followed by a significant elevation of anti-inflammatory cytokines, particularly on day 6. On day 6, the clinical condition of patients was evaluated for the possibility of anti-interleukin administration. If the ratio of IL-1β and IL-10 is low and the IL-10 serum concentration is high in severe COVID-19, the benefit of anti-interleukin administration may need to be considered.

Abbreviations

ARDS, Acute respiratory distress syndrome; AUC, Area under the curve; BAL, bronchoalveolar lavage; CI, Confidence interval; COVID-19, Corona Virus Disease 2019; CRS, Cytokine release syndrome; DfSO, Days from symptom onset; IL-, Interleukin; MODS, Multiorgan dysfunction syndrome; RT-PCR, Real-time reverse-transcriptase polymerase-chain reaction; ROC, Receiver operator characteristic; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

Data Sharing Statement

All data underlying the results are available as part of the article and no additional source data are required.

Ethics Approval and Informed Consent

Written informed consent for publication of the participants/patients' details was obtained from the participants/patients/parents/guardian/relative of the participant/patient. The study was performed following the ethical principles expressed in the Declaration of Helsinki. The study was approved by the institutional review boards of the Dr Soetomo General Hospital by registration number of 1953/KEPK/IV/2020.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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