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Association Between Lymphocyte Level and Severity of Coronavirus Disease 2019 Patients: A Cross-sectional Study in Indonesia

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Abstract

BACKGROUND: Coronavirus disease 2019 (COVID-19) pandemic has caused significant health problems globally. COVID-19 should be considered a systemic disease since it involves multiple systems, including the hematopoietic system and the immune system.

AIM: This study sought to determine the relationship between the lymphocyte levels and the severity of COVID-19 patients in Indonesia.

METHODS: A cross-sectional study was conducted among COVID-19 patients at Dr. Zainoel Abidin Hospital Banda Aceh Indonesia from August 27, to September 20, 2021. The subjects were recruited using consecutive sampling method and the data were obtained at their admission to the hospital. Chi-squared test was used to assess the association between lymphocyte levels and the severity of patients.

RESULTS: A total of 280 COVID-19 patients included of which 56.9% (91/160) of the patients with moderate severity, 76.1% (51/67) with a severe condition, and 84.9% (45/53) with critical severity had lymphopenia. There were no patients with lymphocytosis found in this study (0%). The Chi-squared test suggested that the lymphocyte level was significant associated with the severity of COVID-19 patients with $p < 0.001$.

CONCLUSION: Our study suggests that the lower the lymphocyte level, the higher the severity of COVID-19 patients. The level of lymphocyte is therefore potentially to be used as predictor for the disease severity and needs to be monitored regularly in COVID-19 patients.

Introduction

Coronavirus disease 2019 (COVID-19) pandemic has caused significant health problems globally and is potentially to cause persistent symptoms among the survivors [1], [2]. In Indonesia, the first COVID-19 emergence was reported in March 2020 [3] and has caused disruption in many health systems in the country [4]. The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the virus that causes the COVID-19, occurs through multiple transmission routes, especially through the respiratory system of an infected person and droplets [5], [6]. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter into human cells [7]. After its attachment to the epithelial cells in the respiratory system, the virus begins to multiply and migrates into the airways where it penetrates the epithelial cells.

COVID-19 has 3–14 days of an incubation period. During the course, if infection of SARS-CoV-2 many patients experienced fever, shortness of breath, worsened pulmonary lesions, and decreased lymphocytes [8]. Lymphocytes are a type of leukocyte or white blood cell that protects the body against infectious organisms [9]. Lymphocytes belong to the group of immunocytes or cells that produce immunity. Lymphocytes account for approximately 20–25% of all leukocytes [10]. There are two types of lymphocytes, B and T lymphocytes. B and T lymphocytes are responsible for humoral and cellular immunity, respectively [11]. A rapid increase in pro-inflammatory cytokines production during SARS-CoV-2 infection such as interleukin (IL)-6 plays a role in the decreasing lymphocyte levels mechanism or lymphopenia in COVID-19 patients. Increased levels of ILs (IL-6, IL-2, IL-7, and TNF-α) indicate the occurrence of cytokine storm which might trigger lymphocyte apoptosis. Substantial activation of cytokine has also been associated with lymphoid organs atrophy, including the spleen, and impaired lymphocyte turnover. At this point, lymphopenia or a significant decrease in lymphocyte levels becomes apparent [12]. This is a sign of cellular immunity impairment, which could be related to the disease severity and patient's prognosis [13].

Based on the severity of the condition, COVID-19 is classified as asymptomatic, mild, moderate, severe, and critical [14]. There was no increase in proinflammatory chemokines and cytokines in patients with mild COVID-19 symptoms; in contrast, significant increase of these chemokines and cytokines was observed in patients with severe symptom accompanied by acute respiratory distress syndrome (ARDS) [15], [16]. In addition, cytokine storms occur more frequently in patients with severe to critical symptoms [17]. The more severe the symptoms, the higher the level of chemokines and proinflammatory cytokines, leading to lymphocyte apoptosis. Apoptosis or functional exhaustion of cytotoxic lymphocytes has been reportedly associated with viral development [18]. Another investigation suggested that low lymphocyte levels were associated with biological markers of inflammation, the severity of pneumonia, and prolonged hospitalization [19]. This study sought to determine the relationship between the lymphocyte levels and the severity of COVID-19 patients in Indonesia.

Methods

We conducted a cross-sectional study at Dr. Zainoel Abidin Hospital Banda Aceh, Indonesia from August 27, to September 20, 2021. Patients who were confirmed positive for COVID-19, inpatients at Dr. Zainoel Abidin Hospital during the period of March 2020 to January 2021, and had laboratory results of lymphocyte levels examination at the time of hospital admission were included in the study. All patients admitted to the hospital during those periods were included. This study has been approved by the Health Research Ethics Committee of Universitas Syiah Kuala (232/EA/FK-RSUDZA/2021) and registered in National KEPPKN (1171012P).

Patients' demographic data (age and gender) and the comorbid collected. Age was classified into five age groups (26–35, 36–45, 46–55, 56–65, and >65 years). The comorbid collected were diabetes mellitus, hypertension, coronary heart disease, heart failure, chronic obstructive pulmonary disease, acute kidney injury, chronic kidney disease, and others. The lymphocyte levels of patients at hospital admission were collected. The level of lymphocyte then classified as normal, lymphopenia, and lymphocytosis. The severity of the patients were assessed based on WHO criteria. The severity of the COVID-19 was classified into: moderate, severe, and critical.

The Chi-squared test was utilized to evaluate the association between lymphocyte levels and the severity of patients. In addition, the data normality of lymphocyte levels was tested using the Kolmogorov-Smirnov test and the Spearman correlation was used to analysis the correlation between lymphocyte level and the severity. A $p < 0.05$ was considerate as significant. The analyses were conducted using SPSS version 20.

Results

A total of 280 COVID-19 patients were enrolled in this study and the patients' characteristics are presented in Table 1. Almost a third (31.4%) of the patients aged between 46 and 55 years and represented predominantly by male (60%). More than a half (55.5%) of the respondents had comorbidities. Diabetes mellitus was the most common comorbidity (23.9%), followed by diabetes mellitus with hypertension (11.4%), and hypertension (11.1%). Other comorbidities such as malnutrition, dyslipidemia, dyspepsia, and upper gastrointestinal bleeding were also found among the patients.

Table 1: Demographic data, lymphocyte level, disease severity of COVID-19 patients (n = 280)

Characteristic	Frequency	Percentage
Age (year)		
26–35	18	6.5
36–45	49	17.5
46–55	88	31.4
56–65	72	25.7
>65	53	18.9
Gender		
Male	168	60.0
Female	112	40.0
Comorbid		
With Comorbid	154	55.0
Diabetes mellitus	67	23.9
Hypertension	31	11.1
Diabetes mellitus and hypertension	32	11.4
CHD	6	2.1
Heart failure	4	1.4
AKI	3	1.1
CKD	2	0.7
Heart failure and AKI	2	0.7
COPD	1	0.4
Other comorbid	6	2.1
Without comorbid	126	45.0
Lymphocyte level		
Lymphopenia	187	66.8
Normal	93	33.2
Lymphocytosis	0	0.0
COVID-19 severity		
Moderate	160	57.1
Severe	67	23.9
Critical	53	18.9

CHD: Coronary heart disease, AKI: Acute kidney injury, CKD: Chronic kidney disease, COPD: Chronic obstructive pulmonary disease.

Out of total patient, 187 (66.8%) had lymphopenia and 33.2% (93/280) had normal lymphocyte level (Table 1). There were no patients with lymphocytosis. The mean of lymphocyte levels based on the severity of COVID-19 is illustrated in Figure 1. Our data indicated that the majority of COVID-19 patients with moderate severity had lymphocyte levels ranging from 1,051 to 1,859 cells/mm^3. Meanwhile, most of the patients with severe and critical severity had lymphocyte levels ranging between 685.8 and 1,412 cells/mm^3 and 618 to 1,421 cells/mm^3, respectively. The range of the patient's lymphocyte level decreased as the severity of the patients increased.

In this study, only patients with moderate, severe, or critical severity were admitted to the hospital.

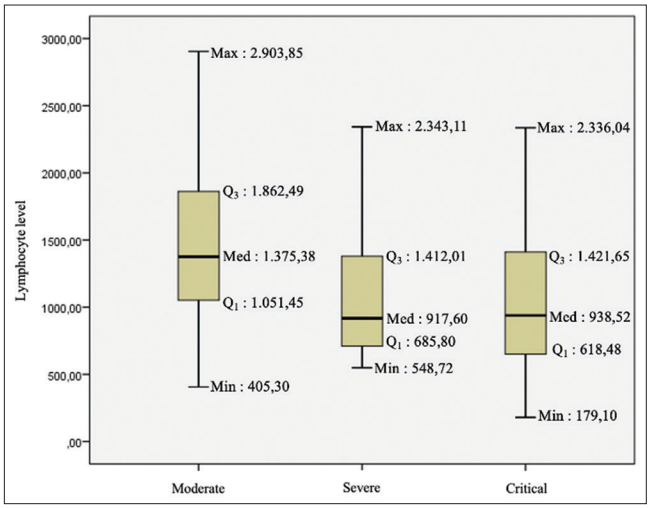

Figure 1: The ranges of lymphocyte levels based on the COVID-19 severity (n = 280)

Therefore there were no mild patients included in the study. Of the total 280 patients, 160 (57.1%) had moderate severity, while 67 (23.9%) and 53 (18.9%) individuals exhibited severe and critical conditions, respectively (Table 1).

The relationship between lymphocyte levels and the severity of the COVID-19 patients is presented in Table 2. The result suggested that lymphopenia occurred predominantly among patients with moderate severity (91 patients) since the distribution of COVID-19 patients treated at Dr. Zainoel Abidin Hospital during the period of March 2020–January 2021 were mostly in moderate severity. However, based on the levels of severity, patients with critical severity showed the highest percentage (84.6%) of suffering from lymphopenia. Our finding suggested that there was a significant association between lymphocyte levels and the severity of COVID-19 ($p < 0.001$). The Spearman correlation analysis revealed a weak negative correlation between variables in this study, indicating that the lower the lymphocyte level, the higher the severity of the patients.

Table 2: Relationship between lymphocyte levels and severity of COVID-19 (n = 280)

Lymphocyte levels	COVID-19 severity						p-value
	Moderate		Severe		Critical		
	n	%	n	%	n	%	
Lymphopenia	91	56.9	51	76.1	45	84.9	<0.001
Normal	69	43.1	16	23.9	8	15.1	
Total	160	100	67	100	53	100	

Discussions

Lymphopenia was found in 187 (66.8%) patients during our investigation, which was slightly lower than that reported in previous studies (75.4–75.7%) [20], [21]. Our data suggested that there was a relationship between lymphocyte levels and the severity of COVID-19 patients. The lower the lymphocyte level, the more severe of the patients. Our finding was consistent with that reported by Huang *et al*. revealing that patients with severely ill had lower lymphocyte levels compared to mild cases ($p < 0.00001$) [22]. Potential explanation regarding the association between lymphopenia and severe illness remains to be determined; however, it has been hypothesized that their relationship might be due to the lymphocyte infection, lymphatic tissue destruction, inflammation-associated lymphocyte apoptosis, or lymphocyte suppression by metabolic diseases such as lactic acidosis [22]. It has also been reported that patients treated in the intensive care unit had higher incidence of lymphopenia, suggesting that lymphopenia was related to the severity of the patients [23] including those with acute thrombotic stroke [24]. Furthermore, patients with ARDS possessed significantly lower lymphocytes levels than those without ARDS ($p = 0.001$) [25].

A study confirmed that $CD3^+$ T cells were the major cell type suppressed during COVID-19 [26], suggesting that SARS-CoV-2 is able of infecting cells, especially T lymphocytes. Data indicates that SARS-CoV-2 could cause changes the lymphocyte cells and other immune cells [27]. Lymphocytes express ACE2 receptors on their surface, allowing SARS-CoV-2 infection and cell lysis. In addition, the occurrence of cytokine storm can promote lymphocyte apoptosis and substantial activation of cytokine has been associated with lymphoid organ atrophies, which might slow the lymphocyte turnover. In addition, SARS-CoV-2 could impair the function of T lymphocytes as well as induce their early hyperactivation, leading to a rapid depletion of cytotoxic $CD8^+$ T cells [12].

It is also possible for critically ill patients to develop ARDS, sepsis, and septic shock, which can lead to cytokine storm due to inadequate lymphocyte cells to maintain immunity [28]. The cytokine storm syndrome, indicated by an increase of cytokines and chemokines, can occur in individuals with severe COVID-19 [29]. Cytokines and chemokines play critical roles both in immunity and immunopathology during viral infection. An irregular and exaggerated immune response can lead to immunopathological conditions such as cytokine storm. A cytokine storm has been evidenced to negatively affects the patients' condition [30].

A previous study has shown that TNF-α, IL-6, and other proinflammatory cytokines could cause lymphocyte deficiency [31]. IL-6 and IL-8 were found to be negatively correlated with lymphocyte level, whereas T-cell depletion was associated with a cytokine surge [31]. It is hypothesized that an immunopathological response to SARS-CoV-2 involving cytokine storm and decreased T cells may underlie the disease progression and death [26]. Furthermore, patients with severe COVID-19 may have high blood lactic acid levels, which might inhibit lymphocyte proliferation [31]. As a result, lymphocytes will decline

rapidly and cause severe lymphopenia in critically ill patients. This condition can also be exacerbated by uncontrolled cytokine storms, resulting in a vicious cycle of low lymphocyte levels and cytokine storms that worsen the condition of COVID-19 patients. Altogether aforementioned mechanisms could explain, in parts, how COVID-19 patients develop lymphopenia.

Conclusion

This study reveals that lymphopenia is frequent in patients with moderate and severe COVID-19 at Dr. Zainoel Abidin Hospital Banda Aceh. Our finding also suggests that there is a relationship between lymphocyte levels and the severity of COVID-19 patients, in which, the lower the lymphocyte level, the more severe the disease.

Author Contributions

All authors are contributed equally to the content of the study, including data collection, statistical analysis, and data synthesis.

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