ORIGINAL ARTICLE

CORRELATION BETWEEN LYMPHOCYTE AND CLINICAL SEVERITY OF COVID-19 IN BANDA ACEH, INDONESIA

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic has caused a global health crisis and is a source of increased morbidity and mortality in the world. COVID-19 caused by SARS-CoV-2 affects the proliferation of lymphocyte, but the clinical significance of lymphocyte remains unclear. Therefore, this study aims to analyze the relationship between lymphocyte with the clinical severity of COVID-19. The retrospective study design collected medical records of demographic data, chest X-rays, and laboratory tests at the time of hospital admission for confirmed patients. Furthermore, the severity of the disease was divided into moderate, severe, and very severe degrees. The severity of lung injury was assessed based on the Brixia Score, and the clinical outcome of patients had criteria of recovered and died. This study has collected 100 COVID-19 patients above 60 years (39%) with male gender (66%). The Majority of patients with low lymphocyte levels (51%), severe lung injury (45%), and one-third of the treated cases ended in death (33.0%), and most of them recovered (67%). Furthermore, low lymphocyte levels associated with severe and critically ill severity and died ($p<0.05$). Almost all lung damage was detected in severe and critically ill severity, and Brixia scores were detected high in patients with low lymphocyte levels (51.0%)($p>0.05$). lymphocyte can be an indicator of clinical severity and mortality for COVID-19 patients. This study shows that lymphocyte count may help identify patients with severe disease. Therefore, observation of the lymphocyte count is necessary for the initial screening, diagnosis, and treatment of COVID-19 patients.

Keywords: *Lymphocyte, COVID-19, lung lesion severity, clinical severity, outcome*

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) has become a global public health emergency because early diagnosis and effective treatment are still challenges.[1] This new disease is caused by the Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) virus that affects several organ systems, especially the respiratory tract in humans, similar to Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS).[2] Meanwhile, most profiles of patients have symptoms similar to mild influenza and sometimes are asymptomatic. Some have severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure (MOF), and death.[3] COVID-19 is expected to continue and the need for intensive care should also increase.[4] In the management of the disease, it is very important to quickly identify potentially critical patients to prevent the spreading and reduce mortality in various parts of the world.[4] Finding and identifying patients at risk of severe COVID-19 complications has an important role in the clinic. Several studies reported that the prevalence of severe COVID-19 ranged from 15.7 to 26.1% in hospitalized patients, and these severe cases are generally diagnosed based on abnormal chest computed tomography (CT) findings and clinical laboratory data.[5] Cardiovascular disease is comorbid with the highest risk of severe COVID-19, and hypertension is the number one risk factor associated with poor clinical outcomes in COVID-19.[6] In Aceh, most men were infected with COVID19 and received treatment at the hospital. The most common clinical manifestations are fever, cough, and shortness of breath. Diabetes Mellitus and Hypertension are the most common comorbidities found in COVID-19 patients treated at government hospitals.[7] Therefore, clinical and laboratory markers are urgently needed to predict the severity of COVID-19 and death. Several resources such as personnel, drugs, and respiratory equipment that are primarily needed by critically ill patients can also be allocated. [8]

Many of observations and clinical studies have shown that lymphocyte levels are decreased in most confirmed patients.[9] Meanwhile, lymphopenia can be considered a major marker for severe COVID-19 infection and pneumonia.[10] It is well known that T lymphocytes have a central role in the body's protection against the coronavirus, the clinical course, and the complications.[11] A decreased T cell count is found in about 60% of patients at the onset of SARS-CoV-2 infection.[11] Several studies show that lymphocytopenia may be associated with cytokine storm (lymphocyte count < 1.5 × 109/L) in severe cases and it is associated with a poor prognosis.[12] This inflammatory cytokine storm

causes severe lung damage, respiratory distress, and other organ failures.[13] Furthermore, lymphocyte is a reliable early indicator of SARS-CoV-2 infection and is very helpful in the assessment of disease progression in COVID-19 pneumonia.[14] Several studies have proven a significant correlation in decreasing lymphocyte counts with severe and critically ill COVID-19.[15] Hence, the number of lymphocytes and lymphopenia can be used as a tool to accelerate the identification of COVID-19 patients with severe clinical presentations. Some suggest that without lymphopenia, they may not suffer from COVID-19, but there are still much data to support this.[16] Therefore, this study retrospectively analyzed the relationship between lymphocyte count and the clinical severity of COVID-19 patients in Banda Aceh, Indonesia.

METHODS

Study setting

The study used a retrospective design by collecting medical record data in the Respiratory Intensive Care Unit and New-emerging and Re-emerging Infectious Diseases ward of Regional General Hospital dr. Zainoel Abidin Banda Aceh from June to September 2020. This study was reviewed and approved by the Institutional Review Board of the School of Medicine, Syiah Kuala University, Banda Aceh (297/EA/FK-RSUDZA/2020), and the National Health Research and Development Ethics Commission (KEPPKN) of the Indonesian Ministry of Health (#1171012P). Furthermore, the data collection technique used was probability sampling with a concecutive sampling, and the sample size of this study was calculated based on the number of samples obtained during the study period.[17] Confirmed COVID-19 patients by positive reverse transcriptase PCR examination from nasal swabs, analyzed molecularly in the hospital virology unit according to WHO and aged more than 18 years, were the inclusion criteria. In addition, those with mild symptoms, probable or suspected cases, COVID-19 re-infection patients, those returning home of their own accord, and incomplete medical record data were excluded from the analysis.

Variable definition

Demographic data (gender, age, and occupation), comorbidities such as hypertension, diabetes mellitus (DM), chronic lung disease, clinical symptoms, chest X-ray examination, and laboratory tests of blood samples taken according to standard procedures at the time of hospital admission were recorded on the data collection form. Meanwhile, lymphocyte was obtained by multiplying the percentage of lymphocyte levels with leukocytes. Clinical severity determined in this study includes disease severity, lung severity, and clinical outcomes. The severity of the disease was divided into moderate, severe, and very severe according to WHO guidelines.[18]Mild disease severity was not analyzed because patients with mild degrees were not treated since they can self-isolate at home. The clinical outcomes of patients when they returned from hospital treatment had criteria of recovered and died.

Assessment of lung injury severity

Lung injury was assessed using the Brixia Score based on Chest X-ray examination results.[19] Furthermore, lung was divided into 6 zones, and scores were given based on the abnormalities found. A score of 0 is no lung abnormalities, 1 is interstitial infiltrates, 2 is interstitial and alveolar infiltrates (interstitial dominant), 3 is interstitial and alveolar infiltrates (alveolar dominant). The score analyzed was the accumulated results obtained from the six lung zones, and based on the mean value, the severity of the injury was divided into low, moderate, and severe. The severity of lung damage was assessed by two experienced radiologists and blind to clinical data.

Lymphocyte Count

An absolute blood cell count is a portion of the totals of a blood test. This study defines the lymphocyte count as the lymphocyte level obtained from multiplying the relative lymphocyte level (%) with the total leukocyte (cells/mm^3) available when the initial patient was admitted to the hospital.[20] Flow cytometry was used to count the number of blood lymphocytes in this study.[21]

Statistical Analysis

Exploratory statistical analysis was conducted to assess potential patient characteristics variables including demographics, clinical symptoms, laboratory results, clinical outcomes, and chest x-ray. For statistical analysis, the lymphocyte levels were divided into low, moderate, and high based on the mean of 1.303. The lymphocyte level is low when it is $\leq$1.303, moderate when the range is 1.303>lymphocyte$\leq$ 2000, and high when it is $\geq$2000. Furthermore, the Spearman test was used to assess the relationship between lymphocyte levels and the clinical severity of COVID19 patients. The significance for all analyzed data was α= 0.05, and all statistical analyses were performed using SPSS (Statistical Package for Social Sciences) for Windows version 25.0 (IBM SPSS Inc., USA).

RESULTS

This study involved 100 confirmed COVID-19 patients above 60 years (39%) as the most age group compared to others, and a total of 66% were male. Weakness, fever, cough, and shortness of breath were the four most common clinical symptoms found, and the most common comorbidities found were diabetes mellitus and hypertension by 43% and 34%, respectively. The

severe COVID-19 cases were mostly found at 44%, while moderate and critically ill severity had the same number (28%). This study showed that majority of patients with low lymphocyte levels (51%), severe lung injury (45%), and one-third of the treated cases ended in death (33.0%), and most of them recovered (67%). Table 1

This study used potential markers of vital signs and laboratory tests to assess the clinical status of patients based on univariate analysis (Table 2). Some of them showed that the average patient treated had a small increase in systolic blood pressure, respiratory rate, and leukocytes. However, there was a decrease in peripheral oxygen levels (SaO2) and lymphocyte counts. From the total patients analyzed, the range of lymphocyte values was between 100 and 3120 with a mean of 1303. This study also assessed the relationship between lymphocyte levels and the clinical severity of COVID-19. Thi study showed that there was a significant association between lymphocyte levels and the disease severity of COVID-19 ($p<0.05$). The lower of the lymphocyte level, the more disease severety of COVID-19 suffered by the patients by 45.1% and 39.2%, respectively. High lymphocyte levels were significantly associated with the clinical outcome of recovered (83.3%)($p<0.05$). However, there was no significant association between lymphocyte levels and lung injury severity, almost majority patients with low lymphocyte level had severe lung injury (51%)($p>0.05$). Table 3

Table. 1 General Characteristics of Patients

Characteristics	Number (n)	Percentage (%)
Age		
18-30 Years	6	6,0
31-45 Years	18	18,0
46-59 Years	37	37,0
>60 Years	39	39,0
Gender		
Male	66	66,0
Female	34	34,0
Clinical Symptoms		
Fever	75	75,0
Cough	74	74,0
Shortness of breath	71	71,0
Indigestion	20	20,0
Sore throat	19	19,0
Loss of smell	10	10,0
Comorbidities		
DM	43	43,0
Hypertension	34	34,0
Obstructive lung disease	7	7,0
Lymphocyte level		
Low	51	51,0
Moderate	31	31,0
High	18	18,0
Severity Disease		
Moderate	28	28,0
Severe	44	44,0
Critically ill	28	28,0
Severity of Lung Injury		
Low	22	22,0
Moderate	33	33,0
Severe	45	45,0
Clinical outcome		
Recovered	67	67,0
Died	33	33,0

Table 2. Laboratory parameters upon hospital admission

Parameter	Min.	Max.	*Mean*	SD
Systolic (mmHg)	96,00	194,00	133,28	18,00
Diastolic (mmHg)	57,00	102,00	78,30	9,22
Heart Rate (x/i)	61,00	138,00	96,49	13,96
Respiratory Rate (x/i)	20,00	40,00	25,41	3,74
SaO2 Without O2 (%)	40,00	99,00	85,79	10,81
Leukocytes (per l)	3.300,00	34.200,00	11.248,00	5905,36
Lymphocyte	100,00	3120,00	1303,55	777,58

DISCUSSION

Many factors can affect the severity and clinical outcome of COVID-19 disease.[22] Generally, age and underlying disease are more susceptible to infection with more severe disease, often ending in the clinical outcome of disability and death.[23] Aging causes a decrease in lung function and tissue pathology, and the immune system in the respiratory tract which, when infected, greatly affects the responsiveness and ability to suppress progression. The results showed that age over 60 years (39%) and male gender (66%) were the most treated groups. Following a study conducted by Chen N, et al, age over 60 years and male gender are more likely to receive hospital treatment.[24]

The most common comorbid factors were diabetes mellitus (43%), hypertension (34%), and chronic obstructive pulmonary disease (7%). This is consistent with the analysis on the incidence of stroke, ischemic heart disease, and diabetes mellitus, which are comorbidities with high incidence rates in Indonesia and Aceh.[25] Furthermore, it is consistent with Huang C, et al, where the most common comorbid factors in COVID-19 were diabetes mellitus (20%), hypertension (15%), cardiovascular disease, and chronic obstructive pulmonary disease.[26]

On average, these patients had a slight increase in systolic blood pressure and respiratory rate but no significant change in pulse rate. These results are consistent with previous studies from China in which tachycardia and hypotension were uncommon features, Another study also stated that the deterioration of patients with COVID-19 was more rapid in viral pneumonia than other bacterial pneumonia, generally showing progressively lower oxygen saturation, greater oxygen demand, and fewer abnormalities in other vital signs.[27]

Lymphopenia is one of the main laboratory markers that can be used as diagnostic and prognostic support in patients with COVID-19. This study showed a significant low lymphocyte levels with increasing disease severity and this is following published data.[28][29 Furthermore, a meta-analysis of 1,289 cases showed a significant relationship between decreased lymphocyte count and disease severity. In published studies, the majority of severe cases presented at admission had a total lymphocyte count of <1 × 109/L while non-severe cases tended to have lymphocytes above this level.[30]

The association analysis showed that lymphocyte levels were not significantly associated with the severity of lung injury. This was based on the brixia score, which showed that lung damage was found in almost all severe and critical symptomatic COVID-19 patients. The brixia score was found to be higher in patients with low lymphocyte levels, and this was consistent with Wang x, et al, where pulmonary lesions were significantly associated with lymphocyte counts. These served as prognostic indicators that have warning implications and contribute to clinical intervention in patients.[31]

Clinical recovery was associated with significantly increased lymphocyte levels. Conversely, the higher the level, the better the clinical outcome of Recovery. This suggested that the functional strength of cytotoxic T cells will improve the body's immunity which effectively eliminates viruses and hold up disease progression.[32]

Limitations

This study was limited to retrospective clinical observations from medical record data of patients. Furthermore, it did not use a control group and was conducted in only one study center. Therefore, for more accurate and precise results as well as wider generalization of the findings, studies in several places are needed to obtain a validated result. The history of taking immunosuppressants that can affect lymphocyte levels was not reviewed further in this study.

CONCLUSION

Lymphocyte is an important parameter in the current care of COVID-19 patients. Patients with low lymphocyte levels necessarily need immediate consideration for hospitalization and clinical intervention to prevent morbidity and mortality in Indonesia.

Table 3. Association between lymphocyte levels and clinical Severity of COVID-19

Lymphocyte	Severity							The severity of lung injury							Clinical Outcome				
	Moderate		Severe		Critical		*p-value*	Low		Moderate		Severe		*p-value*	Recovered		Died		*p-value*
	n	%	n	%	n	%		n	%	n	%	n	%		n	%	n	%	
Low	8	15,7	23	45,1	20	39,2	0,001	11	21,6	14	27,5	26	51,0	0,184	30	58,8	21	41,2	0,050
Moderate	9	29,0	16	51,6	6	19,4		5	16,1	12	38,7	14	45,2		22	71,0	9	29,0	
High	11	61,1	5	27,8	2	11,1		6	33,3	7	38,9	5	27,8		15	83,3	3	16,7	
Total	28	28,0	44	44,0	28	28,0		22	22,0	33	33,0	45	45,0		67	67,0	33	33,0	

Conflict of interest
The authors declare no potential conflict of interest.

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