Increased CD4/CD8 T-cell ratio : A risk factor for mortality in patients with coronavirus disease 2019

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ABSTRACT

Introduction: Although CD4 and CD8 T-cells are the main subset of T-lymphocytes, their roles in COVID-19 infection and severity remain unclear. This study aimed to determine the role of increased CD4/CD8 T-cells ratio as a risk factor for cases of 28-days in-hospital mortality in COVID-19 patients.

Materials and Methods: This study employed a prospective cohort design. Inclusion criteria were confirmed COVID-19 cases with a positive polymerase chain reaction report. CD4 and CD8 T-cells absolute counts were measured by flow cytometry. The CD4/CD8 ratio was calculated by dividing the absolute count of CD4 by that of CD8 T-cells.

Results: A total of 85 subjects were followed for 28 days. The mean age of the subjects was 52.64 years, and majority of them were females (51.8%). Twenty-eight (32.9%) subjects died within 28 days of follow-up. Receiver operating characteristics analysis obtained an area under curve of 0.68 with the cut-off value 1.26 with p = 0.005. Kaplan–Meier's analysis obtained Hazard Ratio 2.91 (95%CI 1.377–6.161; p = 0.0052).

Conclusion: Subjects with an increase in CD4/CD8 T-cells ratio >1.26 had a 2.91-times risk of 28 days in-hospital mortality.

KEYWORDS:
CD4/CD8 T cells ratio, T cell lymphocyte, COVID-19, mortality risk, laboratory testing, SARS COV-2

INTRODUCTION

The pathophysiological process of SARS COV-2 infection is not as yet clearly understood, including the underlying death mechanism in COVID-19 patients, which is suspected to be due to the occurrence of a cytokine storm that has a direct impact on cardiac cell damage.[1] Several cytokines whose level increases under the condition include INF-γ, IL- 1β, IL-6, IL-8, IL-12, and TNF-α, which were known to cause acute respiratory distress syndrome (ARDS).[2] Another hypothesis suggests the occurrence of bacterial sepsis as a secondary infection.[3] Several studies have shown traces of SARS-CoV-2 virus in cases of death occurring in several hematological laboratory parameters such as neutrophil to lymphocyte ratio, relative lymphocytopenia, and platelet to lymphocyte ratio through a cytokine storm mechanism.[2,4,5]

Lymphocytopenia was a common finding in COVID-19 patients that may be a critical factor associated with disease severity and mortality.[6] The mechanism of lymphopenia could be explained by several possible theories, including (i) direct viral infection of lymphocytes, (ii) viruses infecting the lymphatic organs, (iii) apoptosis of lymphocytes due to a cytokine storm, and (iv) inhibition of lymphocyte metabolism that suppresses their proliferation.[5] The development mechanism of this laboratory finding remains unclear. The examination of the CD4/CD8 T-cell ratio will provide an overview of the response of the T-lymphocyte subset. This ratio is often employed in monitoring patients infected with the human immunodeficiency virus (HIV). A CD4/CD8 T-cell ratio <1 indicates a weakened immune system as the number of CD4 T-cells decreases and their proportion is replaced by those of CD8 T-cells. The decrease in this ratio illustrates the weak level of patient immunity resulting in an increased risk of infection.[7] On the other hand, an increase in the ratio of CD4/CD8 T-cells indicates an increase in the rapid immune response against viruses.

This study aimed to determine the role of CD4/CD8 T-cell ratio increment as a risk factor for the occurrence of death in COVID-19 patients.

MATERIALS AND METHODS

This study employed a prospective cohort design. The inclusion criteria were confirmed adult COVID-19 patients (based on the results of the COVID-19 polymerase chain reaction [PCR] examination) and having received treatment at Dr. Sardjito Hospital (SH), both as an inpatient or an outpatient. Patients who were pregnant and who had history of impaired immune system were excluded. Subjects were recruited from December 2020 to March 2021, on the first day of confirmed COVID-19 based on their PCR report. Patients agreed to participate in the study by signing an informed consent form either by themselves or their entitled family member. This research received ethical approval from the ethics committee of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada with the approval number KE/FK/0398/EC/2021.

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Table I: Subject characteristics

Parameter	n (%)
Age (year), Median (range)	57 (18–83)
Male	41 (48.2)
Female	44 (51.8)
Severity	
Mild	9 (10.6)
Moderate	28 (32.9)
Severe	47 (55.3)
Critical	1 (1.2)
Length of stay (days), median (range)	12 (2–67)

Table II: Initial laboratory parameters at hospital admission

Parameter	Mean (± SD) / Median (range)
Hb (g/dL)	11.33 (± 2.51)
Leukocyte count (x10^3 cell/µL)	8.54 (2.11–31.37)
Lymphocyte count (x10^3 cell/µL)	1.26 (± 0.65)
Lymphocyte percentage (%)	13 (1.6–37.6)
CD4 cell count (cell/µL)	254 (13–1066)
CD4 cell percentage (%)	30.66 (3.61–68.96)
CD8 cell count (cell/µL)	222 (21–1049)
CD8 cell percentage (%)	23.96 (2.65–52.76)
CD4/CD8 ratio	1.27 (0.26–5.72)

Table III: Clinical outcome and laboratory examination results

Parameter	Survivor N = 57	Dead N = 28	*p*
Age, median (min–max)	51 (18–72)	63 (35–83)	<0.0001
Male, n (%)	22 (53.7)	19 (46.3)	0.0117
Female, n (%)	35 (79.5)	9 (20.5)	
Severity			<0.001
Mild, n (%)	7 (77.8)	2 (22.2)	
Moderate, n (%)	28 (100)	0 (0)	
Severe, n (%)	22 (53.2)	25 (46.8)	
Critical, n (%)	0 (0)	1 (100)	
Length of treatment (days), median (min–max)	13 (10–67)	10 (2–27)	0.0241
Laboratory parameter			
Hb (g/dL), mean ± SD	11.42 (± 2.63)	11.24 (± 2.45)	0.8234
Leukocyte count (x10^3 cell/µL), median (min–max)	8.03 (2.11–31.37)	10.15 (3.69–30.03)	0.0787
Lymphocyte count (x10^3 cell/µL), mean ± SD	1.41 (± 0.69)	0.99 (± 0.47)	0.0075
Lymphocyte percentage (%), median (min–max)	15.2 (4.5–37.6)	7.3 (1.6–32.9)	0.0010
CD4 cell count (cell/µL), median (min–max)	340 (39–1066)	219 (13–869)	0.0033
CD4 percentage (%), median (min–max)	30.32 (9.26–68.96)	31.45 (3.61–43.98)	0.8959
CD8 cell count (cell/µL), median (min–max)	255 (62–902)	109 (21–1049)	0.0016
CD8 percentage (%), median (min–max)	26.87 (7.81–51.39)	18.65 (2.65–52.76)	0.0021
CD4/CD8 ratio, median (min–max)	1.18 (0.35–5.72)	1.51 (0.26–3.95)	0.0136

Blood samples were obtained from the selected subjects (3 mL of the blood in an EDTA tube) within 24 hours of confirmed COVID-19 tests. Complete blood count analysis was performed with Sysmex XN-1000. Blood samples were then examined by flow cytometry using FACS Canto II with the lyse wash principle. The samples were incubated with CD3 FITC, CD4 PE, and CD8 FITC reagents for 15 min in a dark room and read within 2 hours of incubation. The parameters measured included complete blood count and CD4 and CD8 cells count.

The recruited subjects underwent treatment according to the standard of care at SH. The outcome measured in this study was death within 28 days of hospitalization (28 days in-hospital mortality). Follow-up was conducted for subjects for 28 days after being confirmed positive for COVID-19 and the outcome of death was recorded. The outcomes were categorized as survivors or dead. The survivors were subjects who were declared recovered and discharged from SH or being treated within 28 days. Meanwhile, the subjects who died within 28 days of treatment were categorized as dead.

Categorical data was presented in the form of frequency and proportion. Data with normal distribution are presented in the form of mean ± standard deviation (SD). Data with abnormal distribution are presented in the form of the

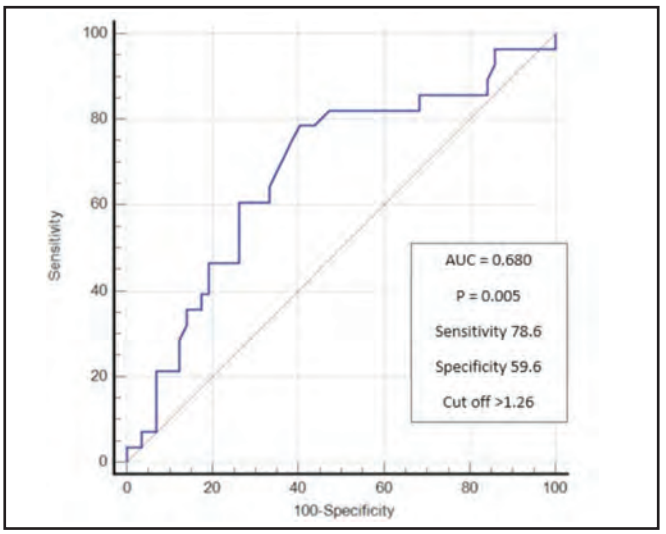

Fig. 1: ROC analysis of CD4/CD8 ratio toward mortality outcome in 28 days of treatment.

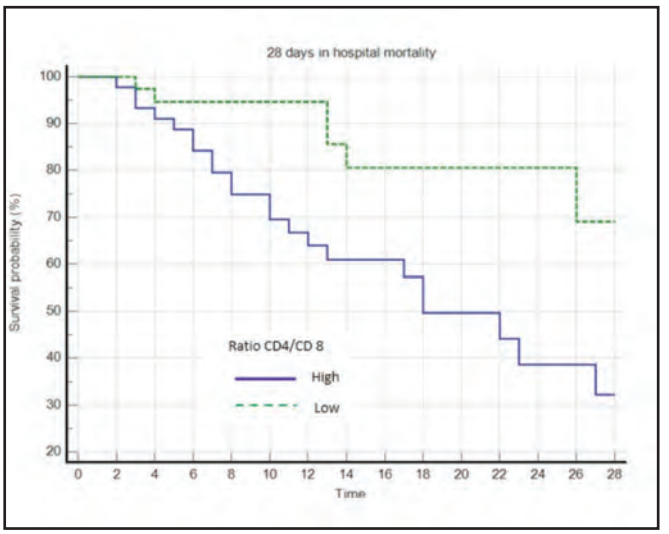

Fig. 2: Kaplan–Meier curve analysis of survival with CD4/CD8 ratio toward mortality outcome in 28 days of treatment.

median (min-max). Statistical analysis was performed by the Receiver Operating Characteristics (ROC) curve to obtain the cutoff value, and Kaplan–Meier analysis was performed to assess the prognosis of death. All statistical analyzes were performed using the Medcalc software, and $p < 0.05$ was considered to indicate statistical significance.

RESULTS

A total of 85 subjects were included in this study. The mean age of the subjects was 52.64 years and majority of the subjects were women (51.8%). A total of 47 subjects (55.3%) were admitted to the hospital with severe conditions. The length of stay at the hospital varied between 2 and 67 days (Table I).

Fifty subjects (65.8%) had a lymphocyte count of <18% with an overall median of 13%. The median percentage of CD4 T-cells was 30.66%, while that of CD8 T-cells was 23.96%. The overall CD4/CD8 T-cell ratio ranged widely from 0.26 to 5.72 (Table II).

Twenty-eight subjects (32.9%) died within 28 days of hospitalization. The fastest length of treatment was 2 days in the dead group and 10 days in the survivor group. There was a significant difference between the groups for the ratio of CD4/CD8 T-cells, with the median in the dead group being significantly higher ($p < 0.05$) (Table III). ROC analysis for the ratio of CD4/CD8 T-cells obtained an area under curve of 0.68, with a cutoff value of 1.26 and $p = 0.005$ (Figure 1). A total of 45 subjects (52.94%) showed a CD4/CD8 T-cell ratio >1.26.

Statistical analysis: proportion difference test using Chi-squared test, difference test with normal distribution using independent t-test, difference test with abnormal distribution using Mann-Whitney test.

The analysis was continued with Kaplan–Meier, obtaining a hazard ratio of 2.91 (95% CI 1.377–6.161; $p = 0.0052$), indicating that the subjects with an increased CD4/CD8 T-cell ratio of >1.26 had a 2.91-times risk of death in 28 days of treatment (Fig. 2).

DISCUSSION

The pathophysiology of COVID-19 in causing cytokine storms remains unclear. The involvement of T-cells in case progression also remains unclear. Preliminary evidence suggests that lymphocytopenia supports the aggravation of the disease.[8] The results of this study showed a similar finding, suggesting that the percentage of lymphocytes decreased in the dead group. These findings confirm the role of T-cells in the pathophysiology of COVID-19.

Previous studies have suggested a correlation between the proportion of a subset of T-cells and disease severity. Lower levels of CD4 and CD8 T-cells were associated with the severe and critically severe groups.[9] The results of this study showed similar findings in the dead group with significant differences.

T-cells, mainly consisting of CD4 and CD8 T-cells, play a major role in the adaptive immune system. T-cells act as a mediator of antibody responses produced by B cells.[10] Pathogenic exposure to CD4 T-cells either directly or mediated by dendritic cells trigger the differentiation of T-cells into their subsets, which then produce interleukin 12. This response ultimately triggers antibody production.[11] In terms of the response to infection with the SARS-CoV-2 virus, this exposure triggers the emergence of antibodies to the virus.[12,13] Likewise, CD8 T-cells that act as cytotoxic can eliminate cells infected with the SARS-CoV-2 virus through the abnormal recognition of MHC class 1.[14,15] The findings in this study indicate a fatigue response of CD4 and CD8 T-cells in patients with aggravation, leading to death.

Deaths that occur in COVID-19 patients are suspected to occur through 2 mechanisms, namely cytokine storm and secondary infection leading to bacterial sepsis.[3,16,17] The results

of this study demonstrated a quantitative decrease in the immune response in the dead group. An exhausted immune system is described by a decrease in the number of CD4 and CD8 T-cells. Although this study did not describe the kinetics of the two subsets of T-lymphocytes, the significant differences between the two groups could justify this point. Past studies conducted on infants of mothers confirmed positive COVID-19 showed normal levels of CD4 and CD8 T-cells in patients with negative results.[18] This finding indicates the kinetics of decreasing levels of CD4 and CD8 T-cells along with the fatigue level of the immune response.

The ratio of CD4/CD8 T-cells is a parameter that can indicate the function of adaptive immunity. This ratio is often used in monitoring patients with HIV to determine the condition of immunity and susceptibility to opportunistic infections.[7,19] In the case of COVID-19, this parameter could be used as a reference to assess the risk of secondary infection. Another study has indicated no significant difference in the CD4/CD8 T-cell ratio between different levels of severity.[9] This study reported that an increase in the ratio of CD4/CD8 T-cells >1.26 had a significantly higher risk of death. This mechanism is suspected in line with the increased risk of secondary infection.

CONCLUSION

This study concludes that CD4/CD8 T-cell ratio >1.26 increases the risk of death of COVID-19 patients by 2.91-times. The limitations of the present study include that it did not measure the kinetics of the CD4/CD8 T-cell ratio and the examination of secondary infections in the subjects. Kinetic data is expected to assist in the analyses of the changes occurring in patients with varying clinical status as well as to confirm the current findings.

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