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Humoral and Cellular Immune Response on COVID-19 Patients and Sinovac Vaccine Participants

Brigitte Rina Aninda Sidharta, Mas Aditya Senaputra

Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Surakarta, Indonesia.
E-mail: rina_aninda@staff.uns.ac.id

ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)-2 is a new SARS-CoV virus. A person who is infected with this virus will induce both humoral and cellular immune responses. Herd immunity can be achieved through vaccination. The purpose of vaccination is the formation of antibodies capable of neutralizing coronavirus against the receptor binding domain. This study aimed to determine the differences between humoral and cellular immune responses between confirmed COVID-19 patients and Sinovac vaccine participants. This observational analytic study with a prospective cohort approach was conducted between March to October 2021. Fifty subjects (25 officers who had received vaccinations for COVID-19 patients and 25 COVID-19 patients treated at the Dr. Moewardi General Hospital) and met the inclusion and exclusion criteria were enrolled. Different tests were carried out to see the difference between the levels of $CD8^+$ T cells and anti-SARS-CoV-2 antibodies in the vaccine group and the COVID-19 patient group. There was no significant difference in humoral immune response (anti-SARS-CoV-2) between the vaccine group and COVID-19 patient group [33.93 (0.4–196.6) U/L vs. 101.28±158.59 U/L; p=0.409], but there was a significant difference in cellular immune response ($CD8^+$) between the vaccine group and COVID-19 patient group [878.52±47368 cells/μL vs. 270.16±213.64 cells/μL; p=0.001]. CD8 assay can be used as a parameter to differentiate the cellular immune response between COVID-19 patients and COVID-19 vaccine recipients.

Keywords: Vaccine, anti-SARS-CoV-2, $CD8^+$

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 is a new SARS-CoV virus that is genetically included in the beta Coronavirus genus, Sarbecovirus subgenus, and 96% identic with bat Coronavirus samples.[1] Virus transmission occurs when particles containing the virus (droplets/ aerosols) are inhaled through the respiratory tract from the coughing or sneezing of an infected person, it can also occur through talking or singing together without wearing a mask.[2]

The N protein holds the RNA genome, while the S, E, and M proteins form the viral envelope. Spike glycoprotein-S facilitates viral attachment to the Angiotensin-Converting Enzyme (ACE)-2 receptor and fusions with host cell membranes.[3] The ACE-2 receptor is the route of entry of the virus into the human body, and has high expression in the lungs, heart, ileum, kidney, and bladder because the ACE-2 receptor is highly expressed in the apical part of the lung epithelial cells in the alveolus, then the virus will damage this section and this is corresponding with the radiological picture, which showed initial lung damage in the distal airways.[4] SARS-CoV-2 then uses serine transmembrane protease serine-2 (TMPRSS2) for the maturation of protein S to infect target cells.[5]

A person who is infected with this virus will induce both humoral and cellular immune responses. In general, viruses that enter the body will be recognized, processed, and presented by Antigen-Presenting Cells (APCs). Upon activation of T cells, cluster of differentiation (CD)4^+ T cells will differentiate into effector T cells and produce cytokines and chemokines, while $CD8^+$ T cells differentiate into cytotoxic T cells and mainly kill viruses that infect target cells. The T cell response to SARS-CoV-2 has a large effect on patient outcomes.[6] Circulating T follicular helper cells (cTfh) increased progressively at 9 days after admission in mild cases and remained in the peripheral circulation throughout the convalescent period, this number being higher than in healthy individuals. The function of Tfh is important for the formation of memory B-cells and the production of high-affinity antibodies. Approximately 19.3% of patients showed a marked increase in neutralizing antibody activity and a decrease 28 days after recovery, indicating that

neutralizing antibodies have a short life span. Liu *et al*. stated that $CD4^+$ and $CD8^+$ decreased in severe COVID-19 infections, but the CD4/CD8 ratio could not differentiate between moderate and severe infections.[7] Wang *et al*. mention that $CD8^+$ increased one week after therapy in patients whose radiological images improved and could be used as a predictor of clinical outcomes compared to $CD4^+$.[8]

Herd immunity can be achieved through vaccination, >90% of people can be immune through vaccination mechanisms. Immunogens used as vaccine candidates include whole viruses (live-attenuated or inactivated), viral vectors, nanoparticles or virus-like particles, subunit components, protein/peptide, RNA, deoxyribonucleic acid (DNA), or live cells. The Corona Vac vaccine developed by Sinovac Biotech China is a vaccine that contains inactivated SARS-CoV-2 virus and uses aluminum hydroxide as an adjuvant. Sinovac contains SARS-CoV-2 strain CN2 extracted from bronchoalveolar lavage (BAL) of patients treated in Wuhan, cultured on Vero cells, harvested, and inactivated using Vero cells propiolactone, purified, and subsequently absorbed into aluminum hydroxide.[9] Inactivated virus vaccines generally only produce antibody-mediated immunity (not cell-mediated immunity).

The purpose of vaccination is the formation of antibodies capable of neutralizing coronavirus against the Receptor Binding Domain (RBD). The vaccine induces T cells and B cells, producing antibodies via $CD4^+$ T cells. The immune response that occurs is intended to prevent infection by inhibiting virus attachment to the ACE-2 receptor. Vaccines train the immune system to identify viruses that cause infection and fight them before the virus has a chance to harm the body by activating $CD4^+$ T cells, which will then stimulate B-cells to produce neutralizing antibodies specific to SARS-CoV-2 and $CD8^+$ T cells to recognize and kill viruses that cause infection present in infected cells.[10,11] Reynold *et al.* mentioned that the COVID-19 mRNA vaccine causes an increase in specific $CD8^+$ T cells, which then differentiate into memory T cells.[12]

Lymphocytes and their subsets ($CD4^+$, $CD8^+$, and Natural Killer/NK cells) play an important role in maintaining immune system function. Lymphopenia occurred in 72% of COVID-19 patients, indicating that in COVID-19 there is impaired immune cell function. A cluster of differentiation 8 concentrations in COVID-19 patients was negatively correlated with inflammatory markers such as Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), and Interleukin (IL)-6, but the $CD4^+/CD8^+$ ratio was positively correlated with inflammatory markers.[13] Cluster of differentiation 4 and $CD8^+$ T cells recognize and react with SARS-CoV-2 antigens causing immune protection, especially reducing disease severity.[14]

This study aimed to determine the differences between humoral and cellular immune responses between confirmed COVID-19 patients and Sinovac vaccine participants.

METHODS

This study was an observational analytic study with a prospective cohort approach to determine "Differences in Humoral and Cellular Immune Responses between Confirmed COVID-19 Patients and Sinovac Vaccine Participants". The research was conducted at the Clinical Pathology Installation of Dr. Moewardi General Hospital Surakarta. The research was conducted from March to October 2021.

The target population in this study were all officers who had received vaccinations for COVID-19 patients who had never been infected with COVID-19 and moderate and severe degree COVID-19 patients treated at the Dr. Moewardi General Hospital from April–June 2021. Inclusion criteria were > 18 years old, male and female, completed Sinovac vaccination, (first and second vaccination), moderate and severe degree of COVID-19 patients, and willing to participate in the study. Exclusion criteria were those who received immunosuppressant therapy, patients with immunocompromised conditions, or patients with malignancy under therapy.

The samples were serum and blood with the anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA). The reagent used was Elecsys anti-SARS-CoV-2 S, which was used to detect antibodies towards SARS-CoV-2 spike (S) protein Receptor Binding Domain (RBD) in serum and plasma (lithium heparin, dipotassium-EDTA, tripotassium- EDTA, and sodium citrate) using the Cobas e411 Roche Diagnostic instrument. Another reagent used is the BD Multitest™ cluster of differentiation (CD)3/CD8/CD45/CD4 which is used to measure the concentrations of $CD4^+$ and $CD8^+$. Using the 8-color FACS Canto instrument, Becton Dickinson (BD) Diagnostic.

The research variable was the antibody to SARS-CoV-2, which reflects the humoral immune response (method principle was double antigen sandwich examination) using the electrochemiluminescence assay (ECLIA) method and $CD8^+$, which reflects the

cellular immune response using the flow cytometry method.

The statistical analysis used was a descriptive analysis of the characteristics of the research subjects. The variables in this study used nominal scale categorical variables, such as age, gender, survivor status, and ratio scale for $CD8^+$ T cells and anti-SARS-CoV-2 antibodies. The normality test of the data used the Shapiro-Wilk if the sample was 50. If the data distribution was normal, then the data was stated as mean±Standard Deviation (SD) and if the data was not normally distributed it was stated as the median (25^{th} percentile–75^{th} percentile). Statistical test for difference tests was performed on the variables of age, gender, leukocytes, % lymphocytes, % neutrophils, NLR, $CD4^+$, and $CD4^+/CD8^+$ ratios using independent T-test for normally distributed data, and Mann-Whitney U-test for data not normally distributed. Difference tests were carried out to see the difference between $CD8^+$ T cells and anti-SARS-CoV-2 antibody levels in the vaccine group and COVID-19 patient group. If the data was normally distributed, the statistical test used was an unpaired T-test, and if the data was not normally distributed the Mann-Whitney test was used.

This research had been approved by the research ethics committee of the Dr. Moewardi General Hospital in Surakarta with ethical feasibility article number: 537/IV/HREC/2021 and the patient's consent or written informed consent. Informed consent was obtained from research subjects. The identity of the patient was kept confidential and all costs associated with the study were the responsibility of the researcher.

RESULTS AND DISCUSSIONS

This research was conducted at the Clinical Pathology Laboratory Installation of Dr. Moewardi General Hospital. Patient samples were taken from the COVID-19 inpatient ward at the Dr. Moewardi General Hospital. A total of 50 research samples were obtained, 25 samples for the COVID-19 patient group and 25 samples for the Sinovac vaccine group. The distribution of data for the lymphocyte parameters of the COVID-19 patient group, the $CD4^+/CD8^+$ ratio of total participants and vaccine group were not normally distributed (using median; minimum, and maximum), while for the parameters age, leukocyte count, total lymphocyte, and vaccine group, neutrophil, NLR, $CD4^+$, and $CD4^+/CD8^+$ ratio of COVID-19 patient group were normally distributed (using mean±SD). All parameters (age, leucocyte count, % lymphocyte, % neutrophil, NLR, $CD4^+$, and

Table 1. Characteristics of research subjects

Variable	Total n=50	Antibody Status Group		p
		Vaccine Group n=25	COVID-19 Patient Group n=25	
Age (years)	43.821±17.39[a]	29.64±5.35[a]	58±13.05[a]	0.001 [$*]
Gender				
Male	27 (54%)	8 (32%)	19 (76%)	
Female	23 (46%)	17 (68%)	6 (24%)	
Corticosteroid treatment				
Yes	25 (50%)	-	25 (100%)	
No	25 (50%)	25 (100%)	-	
Survivor				
Yes	25 (50%)	-	25 (100%)	
No	25 (50%)	25 (100%)	-	
Leucocyte counts (thousand/µL)	7.56±2.79[a]	6.79±1.42[a]	8.32±3.56[a]	0.210[$]
Lymphocyte (%)	22.83±12.29[a]	32±6.94[a]	11.4 (0.71-43.3)[b]	0.001[$*]
Neutrophil (%)	69.55±13.71[a]	59.6±7.45[a]	79.49±11.05[a]	0.001[$*]
NLR	7.58±18.08[a]	2.01±0.73[a]	13.15±24.53[a]	0.001[$*]
$CD4^+$ (cells/µL)	592.54±320.47[a]	822.40±248.92[a]	362.68±194.03[a]	0.001[$*]
$CD4^+/CD8^+$ ratio	1.22 (0.34-4.99)[b]	1.04 (0.59-3.52)[b]	1.74±1.01[a]	0,.002[#*]

Abbreviations: n: number of samples; mmHg: millimeters of mercury; µL: microliter; mg: milligrams; dL: deciliter; COVID-19: Coronavirus Disease-19; NLR: Neutrophil to Lymphocyte Ratio; CD: Cluster of Differentiation

Notes: a: normally distributed data; b: not normally distributed data; [$]Unpaired T-test; [#]Mann-Whitney difference test; *p-value <0.05, significantly significant

$CD4^+/CD8^+$ ratio) in this study were normally distributed, using the mean±SD except for the lymphocyte parameters in the patient group, the $CD4^+/CD8^+$ ratio in the vaccine group and both groups, using the median, minimum, and maximum.

The study subjects were 54% male and 46% female with the mean age in the vaccine group being 26.64±5.35 years and in the COVID-19 patient group was 58±13.05 years.

The corticosteroid treatment was not found in the vaccine group, but on the contrary, all COVID-19 patients received corticosteroids. None of the samples in the vaccine group were survivors.

The mean leukocyte count in the vaccine group was 6.79±1.42 thousand/µL and 8.32±3.56 thousand/µL in COVID-19 patients, there was no significant difference between the two groups with p=1.210.

The percentage of lymphocytes in the COVID-19 patient group was significantly lower (11.4 [0.71-43.3] %) compared to the vaccine group (32±6.49%, p=0.001), differing from the percentage of neutrophils, where the COVID-19 patient group was significantly higher (79.49±11.0%) compared to the vaccine group (59.6±7.4%, p=0.001). There was a significant difference in the NLR between the vaccine group (2.01±0.73) compared to the COVID-19 patient group (13.15±24.53, p=0.001).

The normality test of the anti-SARS-CoV-2 and $CD8^+$ showed not normally distributed data for the anti-SARS-CoV-2 of total participants and the vaccine group, while the anti-SARS-CoV-2 of the COVID-19 patient group and all of the $CD8^+$ were normally distributed.

Table 2 shows the different tests of the research variables of the humoral immune response (anti-SARS-CoV-2) and the cellular immune response ($CD8^+$). There was no significant difference in humoral immune response between the vaccine group [33.93 (0.4–196.6) U/L] and COVID-19 patient group (101.28±158.59 U/L) with p=0.409, but there was a significant difference in cellular immune response ($CD8^+$) between the vaccine group (878.52±473.68 cells/µL) and COVID-19 patient group (270.16±213.64 cells/µL) with p=0.001.

Sinovac vaccination participants samples in this study were taken in the third month after the second vaccination. In Table 2, it can be seen that compared to the vaccine group, the percentage of lymphocytes and $CD4^+$ in the COVID-19 patient group was lower and the percentage of neutrophils was higher when compared to the vaccine group with p=0.001. The Sinovac vaccine only induces a humoral immune response, so only antibodies are produced and they only last for a short period. The mRNA vaccine induces both a humoral immune response and a cellular immune response by increasing CD4 and CD8. Several journals state that after administering the mRNA vaccine, CD8 will differentiate into memory T cells.[15-17]

In severe COVID-19 patients, there was a decrease in all lymphocyte subsets including $CD4^+$, $CD8^+$, NK cells, and B cells. This lymphopenia could be due to an increase in corticosteroids as a result of COVID-19 therapy, increasing circulating neutrophils and monocytes.[18,19] The balance of the number of T cells is regulated through the mechanisms of cell proliferation and apoptosis. In COVID-19 patients, there is a decrease in proliferation caused by IL-10 and an increase in apoptosis.[20,21] The SARS-CoV-2 virus or antigen that enters the body will stimulate cellular and humoral immunity through B and T cells that are specific to the virus. The antibodies produced are IgG and IgM, IgM usually disappears by the end of the 12th week, while IgG can last for a long time and is protective. The immunoglobulin G formed is specific for S or N protein. Several studies have shown that $CD4^+$ and $CD8^+$ cells decreased significantly, and the response to the acute phase was associated with a decrease in $CD4^+$ cells and $CD8^+$ cells.[22] In patients who have recovered from SARS-CoV, $CD4^+$ and $CD8^+$ cells can survive for 4 years and can carry out T cell proliferation, Delayed-Type Hypersensitivity (DTH) response, and IFN- production.[23] There is a relationship between the $CD4^+/CD8^+$ ratio with

Table 2. Characteristics of research variables and different tests

Variable	Total n=50	Antibody Status Group		p
		Vaccine Group n=25	COVID-19 Patient Group n=25	
Anti-SARS-CoV-2 (U/L)	31.59 (0.4–691>2)[b]	33.93 (0.4–196.6)[b]	101.28±158.59[a]	0.409[#]
$CD8^+$ (cells/µL)	574.34±476.09[a]	878.52±473.68[a]	270.16±213.64[a]	0.001[$*]

Abbreviations: SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; CD: Cluster of Differentiation; U: units; L: liters; µL: microliter; n: number of samples; mmHg: millimeters of mercury; µL: microliter; mg: milligrams; dL: deciliter

Notes: a: normally distributed data; b: not normally distributed data; [$]Unpaired T-test; [#]Mann-Whitney difference test; *p-value <0.05, significantly significant

disease severity, there is a significant decrease in the $CD4^+/CD8^+$ ratio in patients with severe and critical disease, as well as in non-survivors.[24] A strong antibody response causes disease severity, while a weak response is related to viral clearance.[19] In pediatric patients, 5 out of 6 children showed a protective humoral response with the appearance of IgG and IgM antibodies against N and S-RBD SARS-CoV-2.[25]

A cluster of differentiation-4 T cells reacting to protein S correlated with anti-SARS-CoV-2 IgG and IgA concentrations in convalescent phase patients. This suggests that T cells help the maturation of B-cells in COVID-19 patients during the recovery period.[26] Vaccines currently being developed contain viral proteins, either in the form of short antigenic peptides or mini genes or in the form of long peptides or full-length viral proteins (DNA/RNA). The Sinovac vaccine is an inactivated viral vaccine in aluminum hydroxide adjuvant, where the antigens in such vaccines are multivalent. Concerns have arisen about the antibodies produced by inactivated viral vaccines. Zhang *et al*. stated in their research that the antibodies that emerged from the inactivated viral vaccine vaccination were lower than the antibodies that appeared due to natural infection (23.8–65.4 vs. 163.7 Geometric Mean Titer/GMT).[9] Table 2 shows that there was no significant difference in anti-SARS-CoV-2 between the vaccine recipient group and the patient group with p=0.409. The average IgG titer produced shows how the vaccine consistently triggers the formation of neutralizing antibodies in vaccine recipients, while natural infection also causes highly variable immune responses.[27]

CONCLUSIONS AND SUGGESTIONS

There were no differences in humoral immune responses between COVID-19 patients and vaccine recipients, but there were differences in cellular immune responses between COVID-19 patients and vaccine recipients. CD8 assay can be used as a parameter to differentiate the cellular immune response between COVID-19 patients and COVID-19 vaccine recipients.

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