Original Research Report

LYMPHOCYTE COUNT AND SARS-CoV-2 ANTIBODY LEVEL IN HEALTHY DONORS' BLOOD AT AN INDONESIAN BLOOD TRANSFUSION CENTER

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus that infects the respiratory system by attacking the mucous and epithelial cells. This infection commonly leads to an increase in lymphocyte count as an immune response to invading pathogens. Moreover, antibodies bind and inactivate foreign substances to destroy pathogens and inhibit their replication. These mechanisms prompt the objective of this study, which was to define the relationship between lymphocyte count and SARS-CoV-2 antibody level. This analytical observational study used a cross-sectional approach with quantitative analysis methods and purposive sampling. Healthy donors who had received coronavirus disease (COVID-19) vaccines provided the samples for this study. A total of 30 blood samples were collected from the Blood Transfusion Center of the Indonesian Red Cross Surabaya Area. This study was conducted in May 2022 at two distinct locations. The examination of lymphocytes was carried out using the flow cytometry method in the Hematology Laboratory, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. In addition, the antibody titer test using the enzyme-linked immunosorbent assay (ELISA) method was performed in the Immunoserology Laboratory of the Surabaya Health Laboratory Center, Surabaya, Indonesia. The analysis revealed an average lymphocyte concentration of 2.2633 x 10^3/µl and an average antibody level of 0.2197 according to the optical density (OD) ratio. The data analysis was performed using Spearman's rank correlation statistical test ($p<0.005$), and the results indicated a lack of significance with $p=0.262$. In conclusion, there is no relationship between total lymphocyte count and SARS-CoV-2 antibody level.

Keywords: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); virus; antibody; lymphocyte; enzyme-linked immunosorbent assay (ELISA)

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Highlights:
1. It is essential to conduct research on SARS-CoV-2 for the purpose of acquiring further understanding, especially concerning the production of antibodies examined using antibody titer blood tests.
2. Although the relationship between the examined variables is not significant, this study offers valuable information on blood test results after the COVID-19 vaccination, which can serve as scientific evidence for further research.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a group of ribonucleic acid (RNA) viruses that originate from animals and can be transferred to humans. The virus can penetrate the body by binding to the receptor angiotensin-converting enzyme 2 (ACE2), which is identifiable by toll-like receptor 3 (TLR3). The toll-like receptor (TLR) activates the natural immune response, which in turn initiates the formation of an adaptive immune response. This includes the stimulation of B cells to differentiate into plasma cells and switch the isotypes of the antibodies produced. TLR3 plays a role in the presentation of viral antigens by dendritic cells to the cluster of differentiation 8^+ ($CD8^+$) cytotoxic T lymphocytes. It also influences the modulation and regulation of T lymphocyte tolerance. In the adaptive immune system, antibodies can function by binding to viral particles and blocking the infection of host cells. T cells have a significant role in recognizing and destroying

virus-infected cells. A history of prior viral infections can increase the ability of effector cells due to the development of immunological memory, which facilitates the activation of recall responses (Farber et al. 2016). However, several studies have demonstrated that the immune response elicited during an infection appears uncontrolled. The hyperactivation of monocytes and macrophages can lead to increased neutrophil activity, upregulation of interleukin 6 (IL-6) expression, elevated levels of C-reactive protein (CRP), and a reduction in lymphocyte count (Laili 2021).

In SARS-CoV-2 infection, lymphocytes function as antigen-presenting cells (APCs) and effectors capable of producing chemokines and cytokines. Lymphocytes can differentiate and proliferate into T helper cells, cytotoxic T cells, or B cells, depending on the stimuli they receive. B lymphocytes can produce two types of antibodies: immunoglobulin M (IgM) that appears during the acute phase of an infection and immunoglobulin-G (IgG) that indicates a persistent infection. IgM antibodies can be identified in the blood serum of individuals who have been infected with SARS-CoV-2 between days 3 and 6 after the infection. On the other hand, IgG can be identified eight days after the onset of symptoms. Seroconversion typically occurs within the second week following the onset of symptoms. Previous studies have suggested that patients who have recovered from SARS-CoV-2 infection can develop long-lasting immunity, characterized by high levels of IgG in the antibody titer. Antibodies present in plasma can be found at reasonably stable levels for at least 5 to 8 months post-infection (Röltgen & Boyd 2021).

Previous studies have demonstrated that lymphocyte counts can serve as an indicator to monitor the treatment and diagnosis of patients with infections. In addition, a decrease in lymphocyte count is the main characteristic for assessing the severity of the disease (Shereen et al. 2020, Lagunas-Rangel 2020). Given the available information, we performed antibody titers to investigate the relationship between total lymphocyte count and antibody level in healthy donors who might have developed antibodies against SARS-CoV-2.

MATERIALS AND METHODS

This study used an analytical observational design with a cross-sectional approach and quantitative analysis methods. Antibody titers were performed to determine the relationship between lymphocytes and SARS-CoV-2 antibodies. The study population consisted of healthy individuals who were donors at the Blood Transfusion Center of the Indonesian Red Cross Surabaya Area. The participants were selected through purposive sampling based on specific criteria, which included healthy individuals who had passed a series of screenings and met the eligibility requirements for blood donation between March and April 2022 (Kesmodel 2018). They also had to be declared healthy and either native citizens or residents of Surabaya, Indonesia. This study excluded individuals who were deemed unqualified as blood donors, ineligible for blood donation procedures, or who came from or resided outside the city of Surabaya (Zetterstrom & Waernbaum 2022). The participants provided blood samples in 3 mL EDTA tubes for lymphocyte counts and in 3 mL plain tubes for antibody titers. Furthermore, the blood donors who had developed antibodies against SARS-CoV-2 were screened using a qualitative antibody test. This study received ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Surabaya, Surabaya, Indonesia, with reference No. EA/841/KEPK-Poltekkes_Sby/V/2022 on 23/3/2022.

The data obtained consisted of primary data with a ratio scale derived from examination results. The blood specimen examinations included antibody titers and lymphocyte counts carried out at two different locations. The flow cytometry method was used to examine the lymphocyte count (Normal values: 18 – 42 x 10 3 /µl) at the Hematology Laboratory, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. Additionally, the enzyme-linked immunosorbent assay (ELISA) method was used in the antibody titer test performed in the Immunoserology Laboratory of the Surabaya Health Laboratory Center, Surabaya, Indonesia. The antibody titer data were assessed by examining the optical density Results higher than the cutoff values of 0.300 optical density were reported as positive for the anti-SARS-CoV-2 antibodies (Clark & Engvall 2018).

Once a ratio scale was derived from the data, the Kolmogorov-Smirnov test was conducted to assess the normality of the data distribution. IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was utilized in the data analysis (Hinton et al. 2014). Following the confirmation of the normal distribution of the data, the investigation proceeded with the Pearson's correlation test. On the other hand, the non-normally distributed data were assessed using the Spearman's rank correlation test (Sedgwick 2014).

RESULTS

This research was carried out by examining total lymphocyte counts and antibody titers using EDTA tubes and plain tubes with a blood sample volume of

3 mL for each type of tube. Table 1 presents the results of the qualitative antibody test conducted on the blood samples to detect their SARS-CoV-2 antibodies.

Table 1. Results of the qualitative antibody screening test of the blood samples.

Sample codes	Examination results	
	IgG	IgM
X070401	Reactive	Non-reactive
X070402	Reactive	Non-reactive
X070403	Reactive	Non-reactive
X070405	Reactive	Non-reactive
X070406	Reactive	Non-reactive
X041101	Reactive	Non-reactive
X041102	Reactive	Non-reactive
X041103	Reactive	Non-reactive
X041104	Reactive	Non-reactive
X041105	Reactive	Non-reactive
X041106	Reactive	Non-reactive
X041107	Reactive	Non-reactive
X041108	Reactive	Non-reactive
X041109	Reactive	Non-reactive
X041110	Reactive	Non-reactive
X041301	Reactive	Non-reactive
X041302	Reactive	Non-reactive
X041303	Reactive	Non-reactive
X041304	Reactive	Non-reactive
X041305	Reactive	Non-reactive
X041306	Reactive	Non-reactive
X041307	Reactive	Non-reactive
X041308	Reactive	Non-reactive
X041309	Reactive	Non-reactive
X041310	Reactive	Non-reactive
X041311	Reactive	Non-reactive
X041312	Reactive	Non-reactive
X041313	Reactive	Non-reactive
X041314	Reactive	Non-reactive
X041315	Reactive	Non-reactive

The specimens used in this study passed the initial screening that was performed using a qualitative antibody test. The test revealed positive results, which indicated that all specimens had an IgG antibody response. In comparison, the specimens did not exhibit a positive IgM antibody response. This might be related to the fact that one of the donor requirements was to have a minimum interval of 3–6 months after the SARS-CoV-2 infection and vaccination.

The results showed that the average value of the total lymphocyte counts in the blood samples collected from healthy donors was within the normal range. The data indicated that the blood donors had favorable levels of physical activity and medication usage. Most of the participants in this study had lymphocytes that fell within the normal range (Table 2). It was because the participants were in good health and were not suffering from or being exposed to infectious diseases.

Tabel 2. Lymphocyte counts and results of the SARS-CoV-2 antibody test.

Sample codes	Lymphocytes (x 10^3 /µl)	Optical density
X070401	2.3	0.2508
X070402	4.3	0.2184
X070403	2.7	0.2667
X070405	3.8	0.1168
X070406	2.3	0.2168
X041101	1.2	0.769
X041102	1.9	0.16
X041103	1.8	0.0866
X041104	1.6	0.1403
X041105	1.6	0.1918
X041106	1.4	0.183
X041107	2.1	0.2012
X041108	1.5	0.2436
X041109	1.9	0.1637
X041110	1.5	0.3373
X041301	1.5	0.3028
X041302	1.9	0.1777
X041303	1.7	0.366
X041304	2.2	0.2142
X041305	2.6	0.1984
X041306	2.7	0.1351
X041307	2.5	0.2563
X041308	2.6	0.292
X041309	3.7	0.3075
X041310	2.2	0.1348
X041311	2.8	0.1066
X041312	2.5	0.1861
X041313	2.3	0.1519
X041314	2.0	0.1091
X041315	2.8	0.1054

Tabel 3. Results of the Spearman's correlation test of lymphocyte count and antibody level.

	Lymphocyte count	Antibody level
Correlation coeff.	-0.211	-0.211
Significance	0.262	0.262

Table 3 shows that there is no significant relationship between lymphocyte count and antibody level variables. The results of the Spearman test on the relationship between lymphocyte count and SARS-CoV-2 antibody level revealed a coefficient of 0.211 with a negative correlation. This indicated that as the antibody level increased, it was followed by a decrease in lymphocyte count.

DISCUSSION

Lymphocytes are a subset of agranulocyte leukocytes, which have various important functions in the immune system, specifically in responding to attacks from microorganisms, foreign macromolecules, and cancer cells. None of the participants in this study exhibited decreased lymphocyte levels, which refers to a level of lymphocytes falling below the normal range due to the pre-existing lymphocytes in the tissue. The decrease in lymphocyte count can be caused by the migration of lymphocytes from the bloodstream to the surrounding tissues (Nourshargh & Alon 2014). The maximum load also causes a reduction in antibody production and a general decrease in lymphocyte function. In this study, it was found that only a single individual (3.33%) exhibited a lymphocyte count above the normal range. The total lymphocyte count might increase due to lymphatic leukemia, mononuclear infection, or viral infection. Elevated levels of lymphocytes may arise in the presence of cellular damage in tissues or organs, necessitating a response to the elimination of the damaged or dead cells (Nisnawati et al. 2021).

During the humoral immune response to SARS-CoV-2, the cluster of differentiation 4^+ T cells ($CD4^+$ T cells) interact with the B cells. The attachment of antigen to receptors on the surfaces of B cells induces the activation and differentiation of B cells into plasma cells that produce antibodies, specifically IgM and IgG (Cox & Brokstad 2020). IgM and IgG antibodies typically appear during the second week post-virus exposure, followed by the emergence of neutralizing antibodies capable of counteracting viral infection. The production of IgM starts to decline in the fourth week and will cease three weeks later. If an individual continues to produce IgM antibodies for more than one month, it suggests that the SARS-CoV virus is still replicating in the body, which is a symptom of an acute stage of disease (Crawford et al. 2021).

Unlike IgM, IgG can remain in the body for an extended period of time. IgG produced in response to SARS-CoV infection can still be detectable up to 24 weeks later. Previous studies have demonstrated that IgG and neutralizing antibodies may persist for up to two years post-infection, which indicates that IgG possesses protective properties against re-infections. According to the research conducted by Ndzouboukou et al. (2021), the decrease in IgG antibody levels approximately three months after SARS-CoV-2 exposure is a natural occurrence within the life cycle of antibodies. Another study revealed a decline in antibody levels over a period of eight months post-infection. The study further showed that there was a slight decrease in T cell counts, although with considerable variations across individuals. The challenging measurement of B cells revealed that the cell counts remained stable despite occasional increases (Hutapea 2022). This suggests that even though there is a decline in antibodies, several components will have been present at sufficient levels. These components can restart antibody production and coordinate immune responses against viruses. The specific mechanisms that lead to post-infection immunologic memory also serve as a foundation for immunity acquired through vaccination.

The production of antibodies may lead to changes in blood components, particularly those related to proteins and white blood cells. Vaccination has been known to increase blood protein levels, leukocyte counts, and erythrocyte sedimentation rates (Kellam & Barclay 2020). Three to five weeks following vaccination, the average levels of white blood cells and protein in the blood will return to their normal ranges. Blood characteristics will likewise return to their original state. However, variations in physical activity, medication, and infection severity can affect the total lymphocyte count (Tiara et al. 2016). On the other hand, antibodies may not form optimally after vaccination due to several factors, i.e., primary and secondary factors. The primary factors include an inaccurate immunization schedule and a history of infection, while the secondary factors consist of age, sex, nutritional status, immune status, and comorbidities (Nisnawati et al. 2021).

Jackson et al. (2020) conducted a comparative study involving unvaccinated coronavirus disease (COVID-19) survivors and vaccinated healthy individuals. It was found that the COVID-19 survivors developed neutralizing antibodies, albeit not to the same level as the vaccinated individuals. If the COVID-19 survivors had received a vaccination, their levels of effective antibodies would have been higher than those of the healthy individuals. Neutralizing antibodies were found to be significantly lower in those who had not been infected but had received a second dose of vaccine than in survivors who had only received one dose of vaccine (Röltgen & Boyd 2021).

Theoretically, as one gets older, fewer naïve T cells are available to respond to vaccinations. The standard ratio of cluster of differentiation 4 (CD4) to cluster of differentiation 8 (CD8) cells rises tremendously because CD8 T cells significantly decrease with age. Aging causes a decline in the T cell receptor diversity of CD8 and CD4 cells, which subsequently results in decreased T cell survival. A shift in the generation of short-lived effector T cells, rather than memory precursor cells, is one of the qualitative changes that can impair follicular T cell helper responses to vaccination. In old age, B cell

counts remain relatively stable, but fewer functional antibodies are produced due to decreased expression of certain proteins (Cheng et al. 2021).

Women have higher activity levels of cytotoxic T cells and lymphocytes compared to men. This includes the expression of proinflammatory and antiviral genes that are upregulated in T cells. Several non-specific indicators of cell-mediated immunity have been demonstrated to increase in women, suggesting that they experience higher lymphocyte proliferation and immunological sensitivity to foreign substances. Generally speaking, women consistently have higher basal levels of immunoglobulins and antibody responses to vaccinations compared to men (Klein et al. 2015).

The relationship between obesity and the immune response in humans has been documented in prior studies. It has been demonstrated that a higher body mass index (BMI) lowers immune function and antibody levels post-vaccination. Additionally, an association has been established between a larger abdominal circumference and lower antibody levels in COVID-19 patients (Ross et al. 2020, Pratikstha 2021). Increased inflammatory cytokine production has been linked to obesity. These cytokines include interleukins, interferons, and tumor necrosis factor (TNF), which are indicative of low-grade chronic inflammation and can impair both innate and adaptive immune responses. Lower antibody levels in an immunological response to the SARS-CoV-2 vaccine were associated with a higher BMI considered as obesity, according to a study conducted on Italian healthcare workers (Pellini et al. 2021).

COVID-19 patients with comorbidities are not recommended to receive vaccinations unless under the supervision of the treating doctor. Obesity, diabetes, and cardiovascular disease are among the comorbidities. These conditions worsen the clinical outcome of the COVID-19 infection. One of the salient characteristics of SARS-CoV-2 infection is lymphopenia, which is associated with the severity of the disease. It was found that CD4+ and CD8+ T cells, B cells, and killer T cells were all impacted in lymphopenia patients (Choi & Cheong 2021).

In this study, the administration of various vaccine boosters had an impact on the antibodies that developed in the blood of the donors. The levels of antibodies generally decrease six months after the administration of the primary dose. Therefore, additional doses are required to boost individual protection, particularly in vulnerable populations. According to the circular issued by the Ministry of Health of the Republic of Indonesia (2022), there are two mechanisms of administering booster shots: homologous (in which the type of booster is the same as the primary dose) and heterologous (in which the type of booster differs from the primary dose). These varied mechanisms might result in different levels of antibodies being formed in each individual.

Strength and limitations

The samples in this study were gathered in almost identical conditions, thereby reducing research bias. This study illustrated the outcomes of multiple blood tests conducted after COVID-19 vaccinations. As a result, the data in this study can provide a scientific overview of the public health significance of the COVID-19 vaccinations. However, this was a unicentric study that covered a narrow geographic area. Additionally, the variables examined in this study were limited to total lymphocyte count as well as antibody level with an observation of the optical density of blood samples. Since the subjects were less homogeneous, it was challenging to obtain accurate data on the formation of antibodies by considering the duration of vaccine administration.

CONCLUSION

There was no relationship between lymphocyte count and SARS-CoV-2 antibody level in the blood of healthy donors. The lymphocyte counts and antibody levels of the blood donors were within normal limits.

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Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee of Poltekkes Kemenkes Surabaya, Surabaya, Indonesia, with reference No. EA/841/KEPK-Poltekkes_Sby/V/2022 on 23/3/2023.

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Author contribution

AGP contributed to the conception and design, analysis and interpretation of the data, drafting of the

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