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Complete blood count derived inflammatory biomarkers and the level of anti-SARS-CoV-2 NAb and S-RBD IgG among cancer survivors receiving COVID-19 vaccines

Andhika Rachman [1*], Anggraini Iriani [2], Cosphiadi Irawan [1], Lugyanti Sukrisman [1], Wulyo Rajabto [1], Nadia Ayu Mulansari [1], Anna Mira Lubis [1], Findy Prasetyawaty [1], Rahmat Cahyanur [1], Dimas Priantono [1], Bayu Bijaksana Rumondor [3], Rachelle Betsy [3], Samuel Juanputra [3]

[1] Division of Hematology and Medical Oncology, Department of Internal Medicine, Dr. Cipto Mangunkusumo General Hospital-Faculty of Medicine Universitas Indonesia, Jakarta, INDONESIA
[2] Department of Clinical Pathology, Yarsi University, Jakarta, INDONESIA
[3] Department of Internal Medicine, Dr. Cipto Mangunkusumo General Hospital-Faculty of Medicine Universitas Indonesia, Jakarta, INDONESIA
***Corresponding Author:** andhikarachman@gmail.com

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ABSTRACT

Background: In the era of coronavirus disease 2019 (COVID-19), it is mandatory to identify vulnerable people with cancers as they have impaired immune system that can lead to high mortality. This study analyzes the complete blood count (CBC) derived inflammatory biomarkers and the level of anti-SARS-CoV-2 neutralizing antibody (NAb) and spike protein's receptor-binding domain immunoglobulin G (S-RBD IgG) among cancer survivors.

Methods: A cross-sectional study was conducted in patients with either solid or hematological cancers who had received two-doses of COVID-19 vaccinations within six months.

Results: From 119 subjects, the COVID-19 vaccines demonstrated laboratory efficacy (median NAb=129.03 AU/mL; median S-RBD IgG=270.53 AU/mL). The seropositive conversion of NAb reached 94.1% and S-RBD IgG reached 93.3%. Additionally, the S-RBD IgG had very weak correlation with absolute monocyte count (R=-0.185; *p*-value=0.044). The NAb also had very weak correlation with leukocyte (Kendall's tau-b (τb)=-0.147; *p*-value=0.019), absolute neutrophil count (τb=-0.126; *p*-value=0.044), absolute eosinophil count (τb=-0.132; *p*-value=0.034).

Conclusion: The seropositivity rate of anti-SARS-CoV-2 NAb and S-RBD IgG were significantly high. However, the CBC derived inflammatory biomarkers had poor correlation with anti-SARS-CoV-2 NAb and S-RBD IgG. Thus, anti-SARS-CoV-2 NAb and S-RBD IgG are currently the only reliable markers for measuring the COVID-19 vaccine efficacy which should be widely accessible.

Keywords: COVID-19, vaccine, cancer, complete blood count, inflammatory biomarkers

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is primarily defined as a viral illness caused by novel coronavirus designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is caused by a betacoronavirus (betaCoV), which belongs to a family of viruses that are common in animals and have the potential to cross the species barriers to humans. The ribonucleic acid (RNA) of SARS-CoV-2 encodes for four main structural proteins, including spike (S), membrane (M), envelope (E), and nucleocapsid (N), with the S protein consists of two subunits S1 and S2. The receptor-binding domain (RBD) is comprised within the S1 subunit and has a high affinity binding to angiotensin-converting enzyme 2 (ACE2) receptors. The spike protein binds to ACE2 on host cells and is endocytosed, followed by the fusion of viral and endosomal membranes and the delivery of the viral genome into the cytoplasm. Antibodies that bind to the spike protein, specifically to the RBD, block the attachment to the host cell and neutralize the virus. It has been revealed that the serum and plasma antibodies commonly produce structural proteins (RBD, S, and N) along with the antibodies appearing in a few days to a few weeks after the symptom onset and usually after the viral RNA decreases or no longer detectable. The persistence of immunoglobulin-G (IgG) antibodies has long been established to identify prior infection, which is also beneficial for serological surveys to determine the prevalence of SARS-CoV-2 infection either in selected groups or broader populations [1, 2].

As of 29 July 2022, a total of 572,239,451 COVID-19 cases have been confirmed worldwide, resulting in 6,390,401 deaths. As of 25 July 2022, a total of 12,248,795,623 vaccine doses have been administered [3]. The number of reported COVID-19 cases worldwide is rising continuously despite social distancing effort and infected persons isolation. Mortality rates were

remarkably high among patients with both active cancer and COVID-19, frequently reported to be around 40%. Furthermore, recent studies suggest that immunocompromised patients frequently encounter a prolonged disease course and may present as 'continuous viral reservoirs', thus provides the development of new viral mutations. Therefore, the prevention of COVID-19 infections and the reduction of the disease severity are required [4, 5].

The World Health Organization (WHO) preserves a working document that consist of vaccine development. The COVID-19 vaccines can be divided into 'traditional' approaches (inactivated or live-virus vaccines), RNA, and deoxyribonucleic acid (DNA) vaccines [1]. The primary goal of COVID-19 vaccines is to induce neutralizing antibodies (NAbs) against SARS-CoV-2 Spike protein [6]. The COVID-19 vaccines are based on the use or induction of antibodies known as NAbs which are capable to prevent infection by blocking the viral replication cycle prior to the first virus-directed synthetic event. Myriad NAbs can block the S protein from binding to ACE2 receptors. It has been extensively established that the antibodies play a crucial part in the host protection against viral infections. Antibodies neutralize the viral infection or replication by specifically targeting viral glycoproteins of enveloped viruses such as the SARS-CoV-2 spike (S) protein or the protein shell of nonenveloped viruses. These proteins bind with cellular receptors and membranes to mediate the viral fusion and penetration into the cytosol [7]. The COVID-19 vaccines activate the innate and adaptive immunity and are invented to elicit spike protein-specific antibodies due to the fact that they are proven to be successful in combating the disease [1].

The successful vaccines induce inflammatory response that stimulate the innate immunity. The inflammatory responses are also essential for the development of adaptive immunity [8]. Several studies have showed that innate myeloid cells, such as neutrophils and monocytes, are of interest in defining vaccine signatures. The diverse innate and adaptive immune-cell subsets, including unconventional subsets such as Tγδ lymphocytes, represent a large source of potential biomarkers that could be used to define the signatures of vaccine response [9, 10]. The absolute lymphocyte count (ALC) predicted a protective response to the influenza vaccination in pediatric cancer patients [11]. Therefore, the inflammatory biomarkers derived from complete blood count, such as platelet to lymphocyte ratio (PLR), neutrophil to lymphocyte ratio (NLR), and monocyte to lymphocyte ratio (MLR), have the potential to be the predictive markers for immunogenicity and efficacy of human vaccines. Recent studies revealed a significant association between elevated NLR, PLR, MLR, and illness severity in diseases such as COVID-19 and cancers. To date, the association between NLR, PLR, MLR, and the COVID-19 vaccine antibody have not been established, especially among cancer patients [12-19].

During the era of COVID-19, identifying cancer survivors are crucial as they have impaired immune system and complications that can lead to mortality. The data on vaccinations among cancer survivors requires extra attention because the data is particularly sparse. This study aims to analyze the level of anti-SARS-CoV-2 antibody NAb and S-RBD IgG titer and the CBC-derived inflammatory biomarkers among cancer survivors upon receiving a complete two-dose cycle of COVID-19 vaccines.

MATERIALS AND METHODS

Research Subjects

This study was a multicenter cross-sectional study conducted at Dr. Cipto Mangunkusumo National Central General Hospital and Pondok Kopi Islamic Hospital. This research was performed in accordance with the Declaration of Helsinki. Written informed consent to participate were obtained from all participants. Full anonymity and confidentiality of the data were maintained.

The samples in this study were gathered through consecutive and convenience sampling for six months from October 2021 to March 2022. The included subjects in this study were patients diagnosed with either solid or hematological cancers; aged ≥18 years old; and had received two doses of COVID-19 vaccinations without booster within six months before the evaluation. Patients who already had their COVID-19 vaccine boosters were excluded. Patients who were pregnant and patients with HIV/AIDS, autoimmune disease, and/or acute infection, as well as patients with poor physical status were also excluded.

Eligible patients who agreed to participate in this research were asked to provide written informed consent. Afterwards, subject characteristics, cancer history and medications, comorbidities, and history of COVID-19 vaccination were collected using a guided questionnaire.

Sample Collection and Processing

Peripheral blood was drawn to perform complete blood count (CBC) and anti-SARS-CoV-2 antibody titer evaluation. After the blood samples were gathered, the CBC such as white blood cell (WBC) was measured using Sysmex™ XN-1000 hematology analyzer (Sysmex Corporation, Kobe, Japan). Anti-SARS-CoV-2 antibody titers in the form of S-RBD IgG and neutralizing antibody (NAb) were tested using Mindray™ CL-900i chemiluminescence immunoassay analyzer (Shenzhen Mindray Bio-Medical Electronics Co., China) using its suitable reagents [20]. The output in the form of the antibody titers machine readings (relative light unit/RLU) and the amount of antibody titers (in AU/ml) were analyzed as the result of this study. According to the assay manufacturer, the cut-off for both of SARS-CoV-2 NAb and IgG seropositivity was >10 AU/mL.

Neutrophil-lymphocyte ratio (NLR) was calculated as the absolute neutrophil count divided (ANC) with the absolute lymphocyte count (ALC); derivative NLR (dNLR) was calculated by dividing ANC with white blood cells count (WBC) minus absolute neutrophil cells (ANC); monocyte to lymphocyte ratio (MLR) was calculated by dividing the absolute monocyte count (AMC) with ALC; basophil to lymphocyte ratio (BLR) was calculated by dividing the absolute basophil count with ALC; eosinophil to lymphocyte ratio (ELR) was calculated by dividing absolute eosinophil count (AEC) with ALC; platelet to lymphocyte ratio (PLR) was calculated by dividing the absolute platelet count (APC) with ALC; platelet to white blood cell ratio (PWR) was calculated by dividing the APC with WBC; systemic inflammatory response index (SIRI) was calculated by dividing the multiplication of ANC and AMC with ALC; and systemic immune-inflammation index (SII) was calculated by dividing the multiplication of ANC and APC with ALC. The normal range for WBC, ALC, ANC, AMC, ABC, AEC, APC was 3.50-10.5×10^9/L, 0.90-2.90×10^9/L, 1.70-7.00×10^9/L, 0.30-0.90×10^9/L, 0-0.3×10^9/L, 0.05-0.5×10^9/L, 150-450×10^9/L in adult patients, respectively.

Table 1. Table on top of a column (font size: 9)

Characteristics	Value (n=119)
Age, median (IQR)	51 (11)
Sex, n (%)	
Female	103 (86.55)
Male	16 (13.45)
Cancer classification, n (%)	
Solid	110 (92.44)
Hematology	9 (7.56)
Cancer types, n (%)	
Brain	1(0.84)
Breast	57 (47.9)
Colorectal	2 (1.68)
Gynecologic	30 (25.21)
Head and neck	13 (10.92)
Kidney	1(0.84)
Leukemia	1 (0.84)
Lung	3 (2.52)
Lymphoma	8 (6.72)
Male genitourinary	2 (1.68)
Pancreatic	1 (0.84)
Chemotherapy, n (%)	
Yes	96 (80.67)
No	23 (19.33)
Radiotherapy, n (%)	
Yes	72 (60.5)
No	47 (39.5)
Immunotherapy, n (%)	
Yes	11 (9.24)
No	108 (90.76)
Time since last cancer treatment, n (%)	
≤6 months	32 (26.9)
>6 months	78 (65.5)
No data	9 (7.6)
COVID-19 prior infection, n (%)	
Yes	27 (22.69)
No	92 (77.31)
COVID-19 Vaccine, n (%)	
mRNA-1273	15 (12.6)
BNT162b2	32 (26.9)
ChAdOx1	19 (16)
BBIBP-CorV or CoronaVac	53 (44.5)

Note. IQR: Interquartile range & COVID-19: Coronavirus disease 2019

Reference range of CBC derived inflammatory biomarkers such as the NLR, dNLR, MLR, BLR, ELR, PLR, SIRI, and SII have not been established for cancer survivors [21, 22].

Statistical Analysis

The averages of numeric variables, such as age and inflammatory markers, were presented in the form of median and interquartile ranger/IQR if the variables were not normally distributed. Otherwise, if the variables were normally distributed, they were presented in the form of mean and standard deviation (SD). The correlation between inflammatory markers such as NLR, PLR, and MLR and COVID-19 antibody titers IgG and NAB were done by either Pearson's correlation analysis (for normally distributed data) and Kendall's correlation analysis (for non-normally distributed data) using the statistical package for the social sciences (SPSS) software version 27. If any of the inflammatory markers and/or COVID-19 titers was not normally distributed, they were first transformed to be normally distributed. Afterwards, they were analyzed using Pearson's correlation analysis. Otherwise, if any of the variables could not become normally distributed despite transformation, those variables were analyzed using Kendall's correlation analysis using the original dataset. The strength of correlations of correlations was classified to 0-0.19 as very weak, 0.2-0.39 as weak, 0.4-0.59 as moderate, 0.6-0.79 as strong, and 0.8-1 as very strong [23].

Table 2. CBC derived inflammatory biomarkers & anti-SARS-CoV-2 antibody level

CBC derived inflammatory biomarkers	Averages
WBC×10^9/L, median (IQR)	6.50 (2.80)
ALC×10^9/L, median (IQR)	1.77 (1.07)
ANC×10^9/L, median (IQR)	3.99 (1.69)
AMC×10^9/L, mean (SD)	0.50 (0.06)
ABC×10^9/L, median (IQR)	0.03 (0.03)
AEC×10^9/L, median (IQR)	0.16 (0.13)
APC×10^9/L, median (SD)	292.76 (92.23)
NLR, median (IQR)	2.20 (1.35)
dNLR, median (IQR)	1.59 (0.87)
MLR, median (IQR)	0.28 (0.18)
BLR, median (IQR)	0.02 (0.02)
ELR, median (IQR)	0.08(0.08)
PLR, median (IQR)	152.17 (101.15)
PWR, median (IQR)	42.23 (19.30)
SIRI, median (IQR)	1.06 (0.76)
SII, median (IQR)	0.66 (0.42)
Anti-SARS-CoV-2 antibody level	
S-RBD IgG in AU/mL, median (IQR)	270.56 (658.01)
NAb in AU/mL, median (IQR)	129.03 (225.61)
Seropositive conversion	
S-RBD IgG for SARS-CoV-2 in AU/mL, n (%)	111 (93.3%)
NAb for SARS-COV-2 in AU/mL, n (%)	112 (94.1%)

Note. IQR: Interquartile range; SD: Standard deviation; WBC: White blood cell; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; AMC: Absolute monocyte count; ABC: Absolute basophil count; AEC: Absolute eosinophil count; APC: Absolute platelet count; NLR: Neutrophil-to-lymphocyte ratio; dNLR: Derived neutrophil-to-lymphocyte ratio; MLR: Monocyte-to-lymphocyte ratio; BLR: Basophil-to-lymphocyte ratio; ELR: Eosinophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PWR: Platelet-to-white blood cell ratio; SIRI: Systemic inflammatory response index; SII: Systemic immune-inflammation index; Nab: Neutralizing antibody; S-RBD IgG: Spike protein's receptor-binding domain immunoglobulin G; & SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

RESULTS

The inclusion and exclusion criteria were fulfilled by 121 subjects. However, two subjects were excluded due to incomplete data. As a result, 119 subjects were included in this study. The characteristics of the subjects can be seen in **Table 1**. Some numeric variables, including the age of the subjects, were not normally distributed.

The complete blood count-derived inflammatory biomarkers, anti-SARS-CoV-2 NAb and anti-SARS-CoV-2 S-RBD IgG level as can be seen in **Table 2**. Most of the inflammatory markers of the subjects were not normally distributed. Only absolute monocyte count (AMC) and absolute platelet count (APC) was found to be normally distributed.

As can be observed in **Table 2** and **Figure 1**, the COVID-19 vaccines demonstrated laboratory efficacy, with the median (IQR) of NAb and S-RBD IgG were 129.03 (225.61) AU/mL and 270.56 (658.01) AU/mL, respectively. The cut-off for both NAb and S-RBD IgG level was 10 AU/mL according to assay manufacturer. The seropositive conversion of NAb reached 94.1% and S-RBD IgG reached 93.3%.

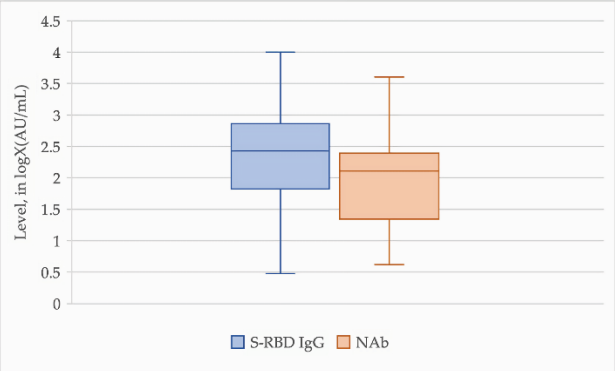

Figure 1. The boxplot of anti-SARS-CoV-2 NAb & S-RBD IgG level, in logX (AU/mL) (Nab: Neutralizing antibody; S-RBD IgG: Spike protein's receptor-binding domain immunoglobulin G; & IQR: Intraquartile range) (Source: Authors' own elaboration)

The vertical axis of the box plot in **Figure 1** represented the values of S-RBD IgG and NAb in the form of logX. The horizontal line in the middle of the box indicated the Q2 value/median of the S-RBD IgG and NAb. The horizontal line on the top and the botttom of the box indicated the Q3/upper and Q1/lower quartile quartile values, respectively. The horizontal lines on the top and bottom of the vertical lines indicated the upper (Q3+1.5×IQR) and lower extreme values (Q1-1.5×IQR), respectively.

Absolute monocyte count (AMC) has very weak to no significant correlation to S-RBD IgG level and Nab level. The leukocyte, absolute neutrophil count (ANC), and absolute eosinophil count (AEC) also had very weak correlation with NAb level. **Table 3** presents the significance, the strength, and the directions of the correlation between inflammatory markers and COVID-19 antibody titers.

DISCUSSION

On a worldwide scale, the progress of the coronavirus disease 2019 (COVID-19) and the SARS-CoV-2 infection has generated a massive burden on healthcare systems, especially among cancer survivors. The primary goal of COVID-19 vaccine is to involve both the memory T-cell and the memory B-cell. The ACE2 with a receptor-binding domain (RBD) is competitively bound by certain neutralizing antibody (NAb) that is produced when B-cell is stimulated. Essentially, this blocks the virus from attaching to cell receptors and entering the cells, thus preventing the infection. The NAb level detection is a significant potential target for the vaccine development [24, 25]. Both NAb and S-RBD IgG have been widely used by researchers to evaluate the vaccine efficacy and determine the optimal vaccine dose [26]. NAb is crucial for viral clearance and is considered to be fundamental for recovery and viral disease protection. A prior study revealed a significant association between the presence of NAb level from prior infections and the decreased rate of reinfection [27-29]. However, the surrogate markers for NAb and S-RBD IgG are needed because NAb and S-RBD IgG measurement is not commercially accessible in clinical settings.

The CBC-derived inflammatory biomarkers were initially suggested as the predictive markers for vaccines immunogenicity and efficacy due to various reasons. First, the inflammatory response is essential to stimulate the innate and adaptive immunity after receiving vaccine [8]. Second, a prior study has showed that absolute lymphocyte count (ALC) predicted a protective response to influenza vaccine in pediatric cancer [11]. Third, the CBC-derived inflammatory biomarkers are inexpensive and widely available in clinical settings. Therefore, our study aimed to analyze CBC-derived inflammatory biomarkers and the level of anti-SARS-CoV-2 NAb and S-RBD IgG among cancer survivors after receiving COVID-19 vaccines.

Table 3. Correlation between complete blood count derived inflammatory biomarkers & anti-SARS-CoV-2 antibody

CBC-derived inflammatory biomarkers	Anti-SARS-CoV-2 antibody (n=119)					
	S - RBD IgG (in logX)			NAb[a]		
	C (Pearson's R)	*p*-value	Interpretation	C (Kendall's tau-b)	*p*-value	Interpretation
WBC (in logX)	-0.146	0.113	No significant correlation	-0.147	0.019	Very weak negative correlation
ALC (in logX)[b]	-0.085	0.358	No significant correlation	-0.093	0.135	No significant correlation
ANC (in logX)[b]	-0.123	0.185	No significant correlation	-0.126	0.044	Very weak negative correlation
AMC	-0.185	0.044	Very weak negative correlation	-0.121	0.053	No significant correlation
ABC (in $[logX]^2$)	-0.088	0.348	No significant correlation	-0.082	0.187	No significant correlation
AEC (in $[logX]^2$)	-0.163	0.078	No significant correlation	-0.132	0.034	Very weak negative correlation
APC	-0.064	0.491	No significant correlation	-0.049	0.427	No significant correlation
NLR (in logX)	0.004	0.969	No significant correlation	0.024	0.699	No significant correlation
dNLR (in logX)	0.300	0.744	No significant correlation	0.032	0.606	No significant correlation
MLR (in logX)	-0.047	0.614	No significant correlation	0.037	0.555	No significant correlation
BLR (in logX)	-0.029	0.755	No significant correlation	-0.034	0.586	No significant correlation
ELR (in logX)	-0.103	0.267	No significant correlation	-0.061	0.327	No significant correlation
PLR (in -1/X)	0.119	0.197	No significant correlation	0.109	0.079	No significant correlation
PWR (in logX)	0.073	0.430	No significant correlation	0.077	0.219	No significant correlation
SIRI (in $-1/\sqrt{x}$)[b]	-0.064	0.493	No significant correlation	-0.028	0.659	No significant correlation
SII (in $-1/\sqrt{x}$)	-0.012	0.901	No significant correlation	0.003	0.963	No significant correlation

Note. [a]Analysis was done without transformation for both NAb & inflammatory markers; [b]One dataset was ignored due to being an extreme outlier; CBC-derived inflammatory biomarker: Complete blood count derived inflammatory biomarker; Nab: Neutralizing antibody; S-RBD IgG: Spike protein's receptor-binding domain immunoglobulin G; WBC: White blood cell; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; AMC: Absolute monocyte count; ABC: Absolute basophil count; AEC: Absolute eosinophil count; APC: Absolute platelet count; NLR: Neutrophil-to-lymphocyte ratio; dNLR: Derived neutrophil-to-lymphocyte ratio; MLR: Monocyte-to-lymphocyte ratio; BLR: Basophil-to-lymphocyte ratio; ELR: Eosinophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PWR: Platelet-to-white blood cell ratio; SIRI: Systemic inflammatory response index; & SII: Systemic immune-inflammation index

We found that seropositivity rate of NAb (94.1%) and S-RBD IgG (93.3%) were significantly high among cancer survivors (**Table 2** and **Figure 1**). The median level of CBC-derived inflammatory biomarkers such as the white blood cells (WBC) count, ALC, absolute neutrophil count (ANC), absolute monocyte count (AMC), absolute basophil count (ABC), absolute eosinophil count (AEC), and absolute platelet count (APC) of the cancer survivors were in normal ranges (**Table 2**). However, up to the writing of this manuscript, the universal laboratory reference values for complete blood count (CBC)-derived inflammatory biomarkers, such as neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocte ratio (dNLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), basophil-to-lymphocyte ratio (BLR), eosinophil-to-lymphocyte ratio (ELR), systemic inflammatory response index (SIRI), and systemic immune-inflammation index (SII), had not been established, especially for cancer survivors. In a cohort study [30], it was reported that the normal value of NLR, MLR, and PLR among Iranian healthy subjects were 1.70±0.70, 11.15±3.14, and 117.05±47.73, respectively. A study showed that conducted among healthy adults in South Korea demonstrated the mean value of NLR, MLR, and PLR were 1.65, 5.31, and 132.4, respectively [31]. Lastly, it was reported that the median value of NLR and PLR were 1.53 and 121.07, respectively [32]. Therefore, further research to determine the most appropriate CBC-derived inflammatory biomarker cut-off values in cancer survivors are warranted.

As can be observed in **Table 3**, the NAb level had a very weak negative correlation with the WBC count (Kendall's tau-b, τb=-0.147, *p*-value=0.019). The WBC count is a nonspecific biomarker for inflammation and is associated with the immune system's response to infection [33]. Several studies demonstrated that the elevated WBC was associated with more critical COVID-19 and cancer prognoses [34, 35]. This finding also was supported by the study [36] who stated that the NAb level also had a poor correlation with WBC and suggested that the production of the NAb did not depend on the number of immune cells.

In this study, the NAb level also demonstrated a significantly very weak negative correlation with absolute neutrophil count (ANC) (τb=-0.126, *p*-value=0.044), which can be seen in **Table 3**. Neutrophils play a critical role in both innate and adaptive host immune responses. Although the neutrophils have an essential role in shaping the adaptive immunity, their function in vaccine-induced immunity toward infection is still unclear [37, 38]. On the other hand, the time of the sampling will be a significant factor on the detection of biomarkers. A prior study demonstrated a correlation between the innate biomarkers and the adaptive responses to the vaccine within 24-hours after vaccination [9]. Nakayama, et al found that the neutrophil infiltration could be detected one month after receiving alum-adjuvanted H5N1 whole virion inactivated vaccines (WIV) [8, 39]. In our study, the blood sample was taken within six months after the two-doses of COVID-19 vaccinations. Therefore, the poor correlation between ANC and NAbs level could be contributed by either the production of NAb that did not depend on the immune response among cancer survivors with immunosuppressive conditions; or the timing of the sampling which was not suitable to reflect the correlation between the NAb level and the immune response to the vaccines.

This study also showed a significantly very weak negative correlation between NAb level and AEC (τb=-0.132, *p*-value=0.034) as can be seen in **Table 3**. Eosinophil is a subpopulation of granulocyte that can mediate immunopathology in eosinophilic diseases, including hypereosinophilic syndromes and bronchial asthma. Eosinophil is reported to have some antibacterial, antiviral, and parasite-protective effects. Remarkably, eosinopenia was present in COVID-19 patients, contradictory to the finding that showed a presence of an association between elevated eosinophil levels and clinical outcome improvement. Eosinophils do not seem to have either a protective or pathogenic role in COVID-19 under normal conditions due to low availability of the data. On the other hand, the SARS-CoV-1 vaccination has been identified to induce pulmonary eosinophilia in mice and monkeys. The reinfection of SARS-CoV-1 in monkeys also induce the eosinophil-associated type 2 inflammation. An infection following RSV vaccination was also associated with a higher rate of eosinophil-associated pulmonary diseases. Furthermore, there is a potential that SARS-CoV-2 vaccinations could result in a similarly vaccine-associated immunopathology [40]. In this study, the poor correlation between NAb level and AEC indicated that the SARS-CoV-2 vaccines were safe without inducing the immunopathology in eosinophilic diseases.

We also observed that prior to the data transformation, the SARS-CoV-2 IgG titer data distribution was not normal among the cancer survivors. This was in line with the findings from prior studies which showed that the antibody response was widely heterogeneous and depended on multiple factors, including the difference of the timing of the sampling and the presence of immunosuppressive conditions among cancer survivors [1, 9]. In **Table 3**, the S-RBD IgG level demonstrated a very weak negative correlation with AMC (R=-0.185, *p*-value=0.044). Furthermore, S-RBD IgG did not have a significant correlation with the other inflammatory biomarkers. The cut-off value for SARS-CoV-2 IgG seropositivity is >10 AU/mL. **Table 2** and **Table 3** showed an efficacy of the vaccines among cancer survivors through the increase of the S-RBD IgG level (median=270.56 AU/mL), survivors which could not be reflected by the complete blood count (CBC)-derived inflammatory biomarker.

Furthermore, the inflammatory biomarkers derived from CBC such as NLR, dNLR, PLR, MLR, SIRI, and SII showed no significant correlation with NAb and S-RBD IgG levels (**Table 3**). These inflammatory markers have been widely used as prognostic markers in COVID-19 and cancer patients, but their roles in vaccinations need further research [12, 41, 42]. One systematic review and meta-analysis showed that increased NLR, PLR, and MLR are significantly associated to illness severity in diseases such as COVID-19 and cancers. However, the association between NLR, PLR, MLR, and antibody response post-vaccination have not been established [15, 16, 18, 19]. Cancer patients have a higher risk to experience immunosuppression than the general population due to a number of factors including impaired immune systems and the use of immunosuppressive therapies which can lead to lymphodepletion and myelosuppression. These conditions could lead to lymphopenia that affect the CBC-derived inflammatory biomarkers including NLR, PLR, and MLR [43-45].

In this study, both NAb and S-RBD IgG levels showed no significant correlation with ALC. This finding was supported by [46] highlighted the same result. However, the correlation between NAb level and ALC remains controversial. Hypothetically, ALC has a significant correlation with NAb

because B-cells as the effector of adaptive immunity produce specific Nab, which then competitively bind to ACE2. ACE2 then bind with RBD, which will prevent the infection by blocking the virus from binding to cell receptors [11, 24].

A recent prospective longitudinal study in patients with multiple sclerosis demonstrated that ALC had a significant correlation with NAb level [47]. Another prospective cohort study in healthy subjects also demonstrated that ALC had a significant correlation with NAb level [48]. To the best of our knowledge, this is the first study, which examine the correlation between the ALC, and the anti-SARS-CoV-2 NAb level among cancer survivors who received immunosuppressive therapy such as chemotherapy, radiotherapy, and immunotherapy, which demonstrated that ALC had no significant correlation with the NAb level (**Table 3**). The cut-off level for SARS-CoV-2 NAb seropositivity is >10 AU/mL. Even though these cancer therapies had immunosuppressive effects, the increase of NAb level, averaging on 129.03 AU/mL, indicated the high efficacy of COVID-19 vaccines among cancer survivors, which was not reflected by the ALC level (**Table 2** and **Table 3**). Thus, we concluded that the anti-SARS-CoV-2 NAb and S-RBD IgG were still the only reliable markers to evaluate the COVID-19 vaccine efficacy.

This study had several limitations. Its cross-sectional nature limited its ability to measure the causal relationship due to the singular time point data sampling. Therefore, large randomized controlled clinical trial employing serial measurements after receiving the primary and booster vaccines, which will more accurately represent the inflammatory responses for the development of adaptive immunity to vaccines, are required in future studies.

CONCLUSION

The seropositivity rate of anti-SARS-CoV-2 NAb and S-RBD IgG were significantly high among cancer survivors. However, the CBC-derived inflammatory biomarker had poor correlation with anti-SARS-CoV-2 NAb and S-RBD IgG level. Thus, the anti-SARS-CoV-2 NAb and S-RBD IgG are currently the only reliable markers for measuring the COVID-19 vaccine efficacy which should be widely accessible. Furthermore, well-powered studies that can overcome the limitations indicated above is fundamental for vaccine design and the potential future course of the pandemic.

Author contributions: AR & AI: conception & design; **CI, LS, WR, & NAM:** interpreted the results; **AML, FP, & RC:** performed the statistical analysis; & **DP, BBR, RB, & SJ:** drafted the manuscript; and Rachman A supervised the study. All authors read and approved the final manuscript. All authors have agreed with the results and conclusions.

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