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Correlation between Soluble Immunosenescence Biomarkers and Serum Neopterin Levels after COVID-19 Vaccination in a Geriatric Population

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Abstract: The geriatric population is increasing worldwide, including in Indonesia which has reached 11.01% in 2021 as the leading country in the geriatric population in Southeast Asia. Consequently, there is an increased risk of having severe infection, malignancy, and autoimmunity. Aging of immunity (immunosenescence) is responsible not only for those conditions but also affects the immune response to vaccination. The study's purpose was to analyze the correlation between immunosenescence and a decreased cellular immune response between 6 and 24 weeks after the second dose of CoronaVac vaccination in the geriatric population. This was an observational analytical study with a prospective cohort design. 73 geriatric subjects (≥ 60 years old) who had already received a second dose of CoronaVac were included. All the subjects had blood taken at 6 and 24 weeks after the second dose of CoronaVac. Serum sCD28, sCD80, and sCTLA-4 were used as immunosenescence markers. The decrease in cellular immunity was measured by the decreasing level of serum neopterin between 6 and 24 weeks following vaccine administration. There is a moderate correlation between sCD28, sCD80, and sCTLA-4 with serum neopterin at weeks 6 and 24. We also found a significant correlation between the decrease in neopterin at 6 and 24 weeks with sCD80 (p = 0.016, r = 0.282) but not with sCD28 (p = 0.078, r = 0.207) or sCTLA-4 (p = 0.017, r = 0.279). sCD80 and sCTLA-4 correlated with a more rapid decrease in neopterin level after CoronaVac vaccination in the geriatric population.

Keywords: Aging; Cellular Immune Response; Neopterin; Soluble Markers; Vaccine

1. Introduction

The geriatric population is increasing globally, including in developing countries such as Indonesia. In 2021, elderly individuals comprised 11.01% of Indonesia's total population of 273.88 million, totalling nearly 30 million people [1]. The increased number of geriatrics is consequently associated with aging in various systems in the human body including the aging of the immune system called immunosenescence. Immunosenescence is characterized by a dysregulation of immune function, which increases the susceptibility to autoimmune diseases, malignancies, and severe infections, while also diminishing responsiveness to vaccination [2]. Immunosenescence is determined by immune risk profiles (IRP). Unfortunately, IRP is difficult to examine since IRP dominantly consists of surface receptors in immune cells and can only be measured with flow cytometry [3]. However, a recent review article indicates that these soluble biomarkers could be used to determine immunosenescence such as soluble CD28 (sCD28), soluble CD80 (sCD80), and soluble Cytotoxic T lymphocyte antigen 4 (sCTLA-4) [4].

CD28 is a receptor that is found in naïve T cells. In geriatrics, the number of naïve T cells is decreased in the immune system in geriatrics since T cells have already received repeated antigenic stimulation [5]. As a receptor of T cells, CD28 has the function to strengthen the transcription effect of the T cell receptor (TCR) also a co-stimulator for naïve T cells [5, 6]. CD28 is expressed by 95% of $CD4^+$ T cells and by 50% of $CD8^+$ T cells. CD28 binds to either CD80 (B7-1) or CD86 (B7-2), receptors on antigen presenting cells (APCs), for activating T cells. Binding of CD28 with CD28 ligand induces anti-apoptotic effects, increases cytokine secretion especially IL-2, upregulates cell adhesion, downregulates T cell anergy, and induces the development of germinal centres [7,8]. Reduced levels of CD28 lead to dysregulation of T cell proliferation, changes in immunoglobulin class, disruption of germinal centre development, and dysregulation of T helper 2 response [9,10]. CD28 is found only on naïve T cells, meanwhile highly differentiated T cells lack the CD28 receptor [5,10]. After CD28 binds to its ligand, either CD80 or CD86, CD28 will shed, become a soluble form or be called soluble CD28 (sCD28) [4,11]. Either the membrane-bound form or soluble form of CD28 has the same function as a T cell regulator. In vitro, sCD28 functions to induce T cell proliferation, IL-6 secretion, and TNF-α. In vivo, sCD28 functions as a marker for increased CD28 expression on T cells, indicating APC and T cell activation. In addition, sCD28 also acts as a molecule that can compete and disrupt the interaction between CD28 or CTLA-4 with the B7 receptor [4]. In various diseases, the role of sCD28 has not been well known, but sCD28 has been found to increase in various autoimmune diseases and inflammation [4,12]. It is known that immunosenescence occurs in these autoimmune diseases, and although increased sCD28 levels have been found in autoimmune diseases, no research has been reported on sCD28 levels associated with immunosenescence in geriatrics.

CD80 is one of the B7 receptors in antigen-presenting cells (APCs) that can interact with CD28 or CTLA-4 receptors in T cells. CD80 is a costimulatory molecule that functions to activate T cells and also regulates B cell activity [13]. CD80 functions to activate T cells by binding to CD28 or suppress T cells by binding to CTLA-4 [14]. The soluble form of CD80, sCD80, is derived from spliced mRNA or the release of the cell surface CD80 receptor into the circulation [15]. sCD80 is thought to function like membrane-bound CD80, namely binding to CTLA-4 and causing T cell suppression or activating T cells by binding to CD28. Research by Horn et al found that in vivo sCD80 functions to activate tumor-reactive T cells and activate the CD28 and TCR transduction pathways [16]. A study found that increasing sCD80 levels will increase IFN-γ production by activated T cells [17]. Elevated sCD80 levels can be used as a marker of ongoing immune system activation. SLE and leukemia patients have elevated sCD80 levels [4]. Another clinical study found elevated sCD80 levels in a large proportion of patients with chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Elevated sCD80 levels correlate with a poor prognosis in CLL and are accompanied by decreased platelet counts and hemoglobin levels, as well as increased leukocyte counts [17]. However, no studies have been reported linking sCD80 levels to immunosenescence and vaccine response in geriatric patients.

Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) is a receptor found on regulatory T cells (Treg) and plays a crucial role in immune system regulation, specifically immune suppression and the prevention of autoimmunity [18]. CTLA-4 is homologous to CD28 and binds to the same ligands, CD80 and CD86, on APCs. CTLA-4 competes with CD28 for binding to CD80 and CD86. CTLA-4 is expressed by activated T cells and B cells. In vitro, the soluble form of CTLA-4, sCTLA-4, is produced by monocytes upon IFN-γ stimulation. In vivo, sCTLA-4 is formed from alternatively spliced mRNA [19]. sCTLA-4 can disrupt the interaction between B7 molecules on APCs and membrane-bound CTLA-4, resulting in impaired T cell suppression. However, sCTLA-4 can also disrupt the inter-

action between B7 molecules and CD28, thus impairing T cell activation. Elevated levels of sCTLA-4 have been observed in various autoimmune diseases, such as Graves' disease, Hashimoto's thyroiditis, myasthenia gravis, SLE, and systemic sclerosis [4]. sCTLA-4 has been found to inhibit the secretion of IFN-γ, IL-2, IL-7, and IL-13, while enhancing the secretion of TGF-β and IL-10 [20]. Furthermore, anti-CTLA-4 antibodies capable of binding to both CTLA-4 and sCTLA-4 have been shown to elicit anti-tumor responses and have been proposed as an alternative therapy for melanoma [17]. These findings suggest that high levels of sCTLA-4 may contribute to the development of malignancies. However, research linking sCTLA-4 to immunosenescence in the elderly population has not yet been conducted.

These three surface receptors—CD28, CD80, and CTLA-4—are shed upon interaction between the T cell and APC, thereby converting into their soluble forms. Upon T-cell activation, the T-cell CD28 receptor interacts with the CD80 receptor on the APC. Following this interaction, both CD28 and CD80 undergo shedding, resulting in their soluble forms. On the other hand, when T-cell activation is no longer required, CTLA-4 expressed on T cells binds to CD80 to induce cell anergy [4,21–23]. Increased levels of sCD28, sCD80, and sCTLA-4 indicate that there is increased activation of T cells that is often encountered in various autoimmune diseases [12], but never studied in geriatric individuals. Consequently, although soluble CD28, CD80, and CTLA-4 are constitutively present in healthy individuals, an elevation in these biomarkers may serve as an indicator of chronic T-cell activation, reflecting the progression toward T-cell senescence. However, in autoimmune disease, there is aging of the immune system similar to geriatrics. This is caused by the continuous activation of the immune system since the self-antigen is known as a foreign antigen [24]. Not only in autoimmune disease, increased in these markers are found in various chronic diseases such as malignancy and chronic infection. Cancerous cells will activate the immune system continuously, thus the immune response will be exhausted [25]. Similar to the mechanisms observed in autoimmunity and malignancy, persistent pathogen exposure during chronic infection exhausts the immune system, thereby accelerating immunosenescence [26]. sCD28, sCD80, and sCTLA-4 can interfere with the interaction of membrane bound CD28 or CTLA-4 T cells with CD80 or CD86 receptor in APC. This means that chronic activation of T cells causes exhaustion of T cells, thus increasing levels of sCD28, sCD80, and sCTLA-4 [4,23].

Since there is no gold standard therapy for treating COVID-19, the most important strategy is preventive measures, one of which is vaccines. Vaccines have become one of the key strategies to prevent disease, as well as to reduce morbidity and mortality. Geriatrics with reduced immune function are prone to severe COVID-19 [27]. Thus, vaccines become more important. However, immunosenescence dampens the immune response, including the immune response to vaccination [28]. Several studies already mention that there is a reduction of humoral immune response following vaccine administration in the geriatric population compared to young adults [29–31]. However, studies about the performance of cellular immune response following vaccine administration are limited.

Several types of COVID-19 vaccines have been produced, including inactivated viruses, viral vectors, and nucleic acids. However, in Indonesia the most common vaccine that has been used is Coronavac, an inactivated virus vaccine. The Indonesian government ordered 271 million doses of vaccine, with the largest number, 125.5 million is CoronaVac. The Indonesian government will provide 30% of the vaccines through the national vaccination program and provide the CoronaVac vaccine free of charge to certain groups, such as healthcare workers, high-risk individuals such as geriatrics, and public servants [32]. Sinovac's CoronaVac vaccine is the most widely administered vaccine to geriatrics in Indonesia and is the most widely used vaccine worldwide, accounting for 50% of all vaccines [33].

Neopterin is one of the pteridine family that is produced by macrophages when stimulated by interferon-gamma. Neopterin is an oxidized form of 7,8-dihydroneopterin, which is a potent antioxidant. Increased neopterin levels are the result of two processes: macrophage activation and the presence of certain oxidants that react with 7,8-dihydroneopterin [34]. Normal serum neopterin values change with age, namely <8.7 nmol/L at ages 19–75 years and <19.0 nmol/L at ages > 75 years [35]. Neopterin has a strong association with cellular immune activity and has been used as a marker of cellular immunity. Increased neopterin levels cause free radical formation, gene transcription, signal transduction, and trigger cell apoptosis [36].

Neopterin has several advantages over classical cellular immunity markers such as interferon-gamma, since neopterin is more stable, has a longer half-time (90 min), and is small and thus can be found in circulation. Meanwhile, interferon-gamma has a shorter half-life (25 min), is produced in the tissue just a few found in circulation in small amounts, and is bound to its receptor thus it is harder to measure the effective level of its biological activ-

ity [34]. A study by Dibakou et al reported that neopterin can be used as a potential biomarker in COVID-19 since it increases in the infected group compared to the healthy group and gradually decreases over time [37]. Another study by Uncu reported that higher neopterin levels are found in the group with a history of COVID-19 and COVID-19 vaccination compared to the group without prior SARS-CoV-2 virus exposure or vaccination [38]. The increase in neopterin levels in people with COVID-19 may be a hint that neopterin levels can be elevated too following vaccine administration with an inactivated vaccine.

This study aims to find the correlation between immunosenescence and cellular immune response following vaccine administration in geriatric individuals.

2. Materials and Methods

This is an observational analytical study with a cohort prospective design study. Subjects are all geriatric (age ≥ 60 years old) and have been vaccinated with a second dose of CoronaVac in Malang, East Java, Indonesia. Exclusion criteria are subjects who have an acute infection or have chronic renal disease when a blood sample is taken. All subjects who agree to be enrolled in this study will sign written informed consent. All subjects had their blood taken two times, 6 weeks following vaccine administration and 24 weeks following vaccine administration. All samples were collected and centrifuged; the serum was stored at −80 °C. Week 6 samples were tested for sCD28, sCD80, sCTLA-4, and neopterin while week 24 samples were tested for neopterin.

Soluble CD28, sCD80, and sCTLA-4 examinations used serum from subjects with the Enzyme-Linked Immunosorbent Assay (ELISA) indirect method. The reagents used were Human sCD28, sCD80, sCTLA-4, and neopterin ELISA Kits from the Bioassay Technology Laboratory. The examination procedure was to prepare all reagents and place the reagents and subjects at room temperature (18–25 °C) naturally for 30 min before starting the examination. Each well was added with 50 µL of standard or 50 µL of subjects. Then, 100 µL of HRP conjugate was added to each well, covered with an adhesive strip, and incubated for 60 min at 37 °C. The plate was washed four times by aspirating the contents of each well and filling each well with wash solution, then re-aspirating each well and discarding it. Washing was carried out up to four times. After the final wash, the plate was flipped and pressed on a paper towel. Then, 50 µL of chromogen solution A and 50 µL of chromogen solution B were added to each well, mixed gently, kept away from light for 15 min and incubated at 37 °C. Finally, 50 µL of stop solution was added to each well and the results could be read with a microplate reader at an absorbance of 450 nm O.D. within 15 min after adding the stop solution.

The normal level of sCD28, sCD80, and sCTLA-4 in adults in this study is 0.83 ± 1.35 ng/mL [23], 0.024–0.318 ng/mL [39], and 5.9 ± 5.4 ng/mL respectively [40]. Data are tabulated in Microsoft Excel for Windows and then statistically analyzed using SPSS 26.0 for Windows. All data are checked for normality test using Kolmogorov-Smirnov. Then, a correlation study using Spearman correlation is performed to see the correlation between soluble markers (sCD28, sCD80, and sCTLA-4) with week-6 neopterin, week-24 neopterin, and decreased of neopterin levels.

Data normality was then tested using the Kolmogorov-Smirnov test. All subjects were tested descriptively for sCD28, sCD80, and sCTLA-4 levels. A correlation test was also performed to determine the correlation between sCD28, sCD80, and sCTLA-4 levels with age and serum neopterin levels. Simultaneous multivariate regression and partial multivariate regression were then performed to determine the greater influence of sCD8, sCD80, and sCTLA-4 on decreasing serum neopterin levels.

This study is approved by the Ethical Committee of the Medicine Faculty of Universitas Brawijaya with number 134/EC/KEPK – S3/06/2023. All participants agreed and signed written informed consent to be enrolled in this study.

3. Results

3.1. Characteristics of Research Subjects

At 6 weeks following vaccine administration, 88 subjects were enrolled in this study. However, 15 of the subjects dropped out 24 weeks following vaccine administration since they had already received a third dose of vaccine or suffered COVID-19 before the blood was taken. The total number of subjects in this study is 73. Characteristics of the subjects are shown in **Table 1**. Of 88 subjects, 40 have no comorbidities, while 48 have comorbidities, consisting of hypertension, diabetes mellitus, dyslipidemia, a history of acute coronary syndrome, and asthma. Mean levels of sCD28, sCD80, and sCTLA-4 in elderly subjects are higher than normal levels in young adults. The Neopterin level

of all subjects in week 6 is significantly higher than in week 24.

Table 1. Characteristics of the subjects.

Variables	N = 73	*p*-Value
Age (years old), mean (min–max)	68.44 (60–94)	
Gender		
- Female, n (%)	40 (54.8)	0.483*
- Male, n (%)	33 (45.2)	
Comorbid, n (%)		
- Hypertension	25 (34.2)	
- Diabetes mellitus	10 (13.7)	
- Dyslipidemia	3 (4.1)	
- History of ACS	4 (5.5)	
- Asthma	2 (2.7)	
- None	28 (38.4)	
sCD28 level (ng/mL), mean ± SD	6.04 ± 4.42	
≤ 2.18 (ng/mL), n (%)	2 (2.74)	
> 2.18 (ng/mL), n (%)	71 (97.26)	
sCD80 level (ng/mL), mean ± SD	2.81± 2.03	
≤ 0.318 (ng/mL), n (%)	0 (0)	
> 0.318 (ng/mL), n (%)	73 (100)	
sCTLA-4 level (ng/mL), mean ± SD	16.89 ± 15.64	
≤ 11.3 (ng/mL), n (%)	24 (32.88)	
> 11.3 (ng/mL), n (%)	49 (67.12)	
Week-6 Neopterin level (nmol/L), mean ± SD	5.64 ± 4.13	0.003**
Week-24 Neopterin level (nmol/L), mean ± SD	4.79 ± 2.86	

Note: ACS, arterial coronary syndrome; SD, standard deviation; **p* value is calculated using the Mann-Whitney test; ***p* value is calculated using the Spearman correlation test and is significant ($p < 0.05$).

3.2. Correlation between sCD28, sCD80, and sCTLA-4 with Age

Table 2 shows that there is a positive correlation between sCD28 and sCTLA-4 with age but not sCD80 with age. This shows that older individuals have higher sCD28 and sCTLA-4 than younger individuals. Meanwhile, sCD80 is not correlated with age. However, all of the subjects have high sCD80 levels compared to normal levels in adults.

Table 2. Correlation between Age and Soluble Markers.

Correlations	*p*-Value	r
Age and sCD28	0.132	0.178
Age and sCD80	0.204	0.150
Age and sCTLA-4	0.009*	0.302

Note: **p* value is significant.

3.3. Correlation between sCD28, sCD80, and sCTLA-4

Table 3 provides a correlation between soluble markers; there is a strong correlation between all three soluble markers. The strongest correlation is between sCD28 and sCTLA-4 while the weakest correlation is between sCD80 and sCTLA-4.

Table 3. Correlation Between sCD28, sCD80, and sCTLA-4.

Correlations	*p*-Value	r
sCD28 and sCD80	< 0.001*	0.736
sCD28 and sCTLA-4	< 0.001*	0.709
sCD80 and sCTLA-4	< 0.001*	0.629

Note: **p* value is significant.

3.4. Correlation between sCD28, sCD80, and sCTLA-4 with Neopterin

Correlation between sCD28, sCD80, and sCTLA-4 with serum neopterin levels is shown in **Table 4** (week-6 neopterin) and **Table 5** (week-24 neopterin). There is a moderate correlation between the three soluble markers and neopterin, both at week 6 and week-24. The decline of neopterin level is calculated by week 6 neopterin level minus week 24 neopterin level. A comparison between week 6 and week 24 revealed a statistically significant

decrease in neopterin concentrations over this 18-week period ($p < 0.05$). This decline in neopterin level is correlated with sCD80 and sCTLA-4 but not with sCD28 (**Table 6** and **Figure 1**). Following a Bonferroni adjustment, the revised statistical significance threshold was established at $p = 0.017$. Consequently, the correlation between sCTLA-4 and the decline in neopterin levels was no longer statistically significant, despite initially demonstrating a conventional *p*-value of less than 0.05.

Table 4. Correlation between Soluble Markers with Week-6 Neopterin Level.

Correlations	*p*-Value	r
sCD28 and week-6 neopterin	< 0.001*	0.565
sCD80 and week-6 neopterin	< 0.001*	0.544
sCTLA-4 and week-6 neopterin	< 0.001*	0.553

Note: *p value is significant.

Table 5. Correlation between Soluble Markers with Week-24 Neopterin Level.

Correlations	*p*-Value	r
sCD28 and week-24 neopterin	< 0.001*	0.624
sCD80 and week-24 neopterin	< 0.001*	0.521
sCTLA-4 and week-24 neopterin	< 0.001*	0.559

Note: *p value is significant.

Table 6. Correlation between Soluble Markers with Decreased Neopterin Level.

Correlations	*p*-Value	r
sCD28 and decreased neopterin	0.078	0.207
sCD80 and decreased neopterin	0.016*	0.282
sCTLA-4 and decreased neopterin	0.017	0.279

Note: *p value is significant.

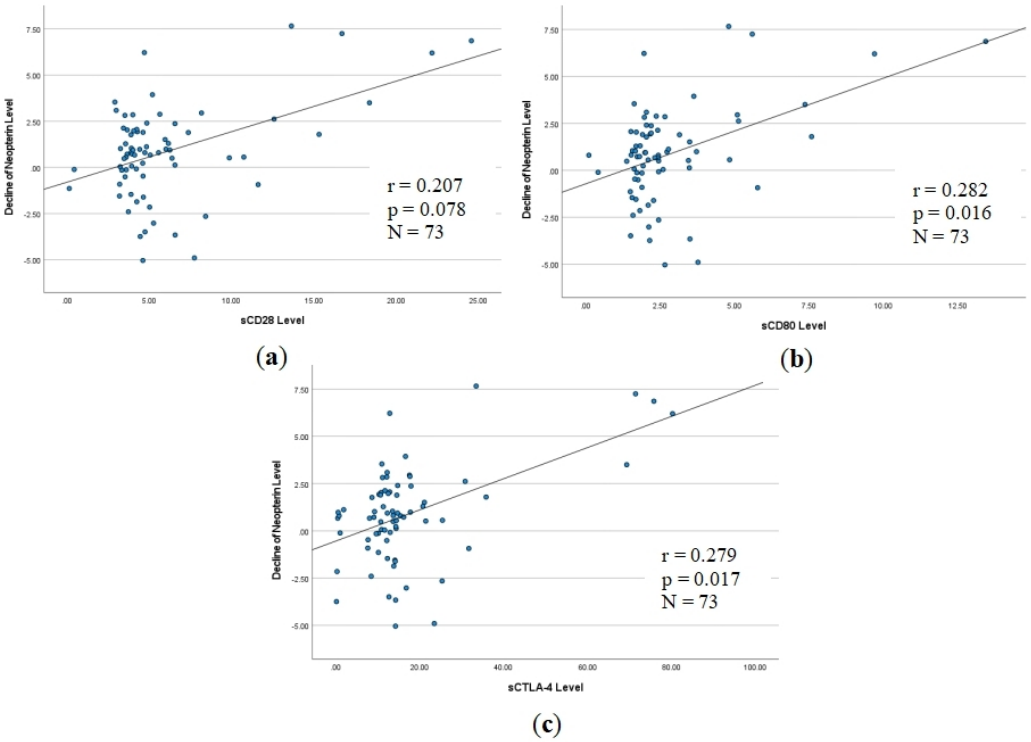

Figure 1. Scatter plot correlation between decline of neopterin level with (**a**) sCD28 level, (**b**) sCD80 level, and (**c**) sCTLA-4 level.

3.5. Multivariate Regression of sCD28, sCD80, and sCTLA-4 towards Decline of Serum Neopterin

The result of simultaneous multivariate regression of sCD28, sCD80, and sCTLA-4 towards decline of serum neopterin (**Table 7**) is significant with $p < 0.001$. The determination coefficient (R^2) is 0.258 (25.8%), meaning that those predictor variables contributed towards 25.8% of the decline of serum neopterin. The other 74.2% are variables from outside the model. After being tested simultaneously, a partial test was carried out to determine which variables were significant to the model.

Table 7. Partial Multivariate Regression of sD28, sCD80, and sCTLA-4 towards Decline of neopterin Serum.

Variables	Regression Coefficient (β)	Standardized Coefficients Beta	*t*	Sig.
sCD28	0.013	0.022	0.060	0.952
sCD80	−0.169	−0.135	−0.466	0.643
sCTLA-4	0.106	0.655	2.464	0.016

The partial test results of sCD28 and sCD80 variables on the decrease in serum neopterin obtained a sig. value > 0.05 so it can be concluded that sCD28 and sCD80 do not have a significant correlation with the decrease in serum neopterin. The partial test results of sCTLA-4 variables on the decrease in serum neopterin obtained a sig. value of 0.016 (<0.05), it can be concluded that sCTLA-4 is associated with the decrease in serum neopterin. Standardized Coefficients Beta shows that the increase in sCTLA-4 levels (0.655) has a greater correlation than sCD28 and sCD80 on the decrease in neopterin levels.

4. Discussion

This study enrolled 73 geriatric subjects who had already received a second dose of CoronaVac. In this study, we found that more than 95% of subjects have elevated sCD28 levels, 100% have elevated sCD80 levels, and 70.45% have elevated sCTLA-4. An elevation in sCD28, sCD80, and sCTLA-4 indicates that there is an accumulation of activated T cells, and these soluble markers could interfere with the interaction between the T cell and APC as a result the immune function will be altered [16,17,22]. We also found that sCD28 and sCTLA-4 are positively correlated with age but not sCD80. In this study, sCD80 is not correlated with age but all of the subjects have high sCD80 levels compared to normal levels in adults. Thus, sCD80 might not exhibit a significant correlation with age in geriatrics but it is significantly higher in geriatrics compared to adults. This study also has a similar result to a study by Handono et al that found an increase of sCD28, sCD80 and sCTLA-4 in the elderly compared to young adults [41]. A study by Zhang et al. demonstrated the role of sCD28 as a biomarker of immunosenescence [42]. Furthermore, while elevated expression of membrane-bound CD80 and CTLA-4 has been documented on senescent immune cells, reports regarding their soluble counterparts remain sparse [43,44].

It is known that increased sCD28 levels in these diseases originate from membrane CD28 shedding [4]. A study reported that increased sCD28 levels were negatively correlated with the number of $CD28^+$ T cells [23]. In addition, a study by Wang et al found that sCD28 levels were negatively correlated with cytotoxic T cell activity [45]. Increased sCD80 levels may originate from membrane CD80 shedding. Membrane CD80 shedding occurs when APC cells interact with T cells with CD28 or CTLA-4 receptors for T cell activation or anergy. High sCD80 levels can occur due to continuous interaction between APC and T cells [4,16]. CTLA-4 is an immune checkpoint molecule that is important in regulating the immune system so that normal body cells are not damaged by immune cells. Increased levels of sCTLA-4 indicate a decrease in T cells with membrane-bound CTLA-4 receptors and are positively correlated with inflammatory markers such as IL-6, IL-1α, IL-1β, TNF-α, IFN-γ, and IL-8, indicating that inflammation-aging can cause increased levels of sCTLA-4 [5]. A study by Kennedy et al found that increased levels of sCTLA-4 inhibit the activity of CD8+ T cells [19]. The increase in these three soluble markers indicates the accumulation of highly differentiated T cells and the presence of chronic inflammatory conditions. In addition, increased levels of sCD28, sCD80, and sCTLA-4 can also disrupt the interaction between T cells and APCs, resulting in changes in immune system function [4].

Moreover, there are strong correlations between these soluble markers. This is in line with a study by Cao et al that these soluble markers are correlated significantly in rheumatoid arthritis patients [46]. A review article by Aprilia et al reported that a study found that there is a positive correlation between sCD28 and sCTLA-4 in systemic

lupus erythematosus patients but not with sCD80. However, there is an increase in sCD28, sCD80, and sCTLA-4 in systemic lupus erythematosus patients compared to healthy subjects. Another study also found a correlation between sCD28 and sCTLA-4 but not with sCD80 in children with allergic asthma. Nevertheless, those markers are all elevated in children with allergic asthma [4]. sCD28 or sCTLA-4 is shed from T cells when T cells interact with APC. Simultaneously, sCD80 is shed from APC. Thus, these soluble markers may be elevated under the same condition and have a strong correlation.

In addition, this study demonstrated that while sCD28 and sCTLA-4 levels were positively correlated with chronological age, sCD80 levels did not display a linear correlation. Notably, however, only a single subject failed to exhibit an increase in sCD80 levels, suggesting that all three markers are broadly associated with the aging process. This finding aligns with data from Handono et al., who reported a significant elevation in sCD28, sCD80, and sCTLA-4 profiles within the older adult population compared to younger controls [41]. Furthermore, these three soluble markers are routinely identified in conditions characterized by chronic inflammation—such as persistent infections, malignancies, and autoimmune disorders—which share mechanistic parallels with immunosenescence [4]. Consequently, the observed age-related associations for sCD28 and sCTLA-4, alongside the elevated sCD80 expression present in 99% of our cohort, correspond closely with established immunological frameworks.

Furthermore, our analysis demonstrated a moderate correlation between sCD28, sCD80, and sCTLA-4 and serum neopterin levels at both week 6 and week 24. Cellular T-cell aging is characterized by altered cytokine dynamics, notably involving interferon-gamma (IFN-γ). Literature indicates that IFN-γ production increases with age, primarily driven by late-differentiated T cells (LDTC), particularly within the CD28− subset. These LDTC subsets typically accumulate during aging, which may contribute to systemic inflammation and an attenuated immune response to novel antigens. This biological shift is supported by animal models, where the majority of CD28− T cells produce IFN-γ, accounting for 68% in aged mice compared to merely 9% in young cohorts [47]. Because elevated IFN-γ concentrations stimulate macrophage activation, this pathway is strongly associated with upregulated neopterin synthesis [37]. Consequently, these findings suggest a potential link between age-related T-cell alterations—reflected by elevated sCD28, sCD80, and sCTLA-4 profiles—and the advancement of inflammaging via neopterin pathways.

In this study, we also analysed the role of immunosenescence in cellular immune response after the vaccine. Cellular immune response was measured with serum neopterin. Neopterin was examined two times, at week 6 and week 24 after the vaccine, thus we can analyse the decline of neopterin levels. We found that there is a correlation between the decline of neopterin levels and sCD80 but not with sCD28 and sCTLA-4 levels. As a result, immunosenescence in geriatrics is associated with the decline of cellular immune response after the vaccine. The positive correlation between sCD80 and a decrease in neopterin indicates that T cell aging is correlated with a more rapid decline in the cellular immune response following vaccine administration. T cell aging causes impaired T cell function and an increase in sCD80 can interfere with T cell activation [4]. Although in immunosenescence there is an increase in basal pro-inflammatory cytokines, there is also impaired function of immune cells including T cells and macrophages which play a significant role in cellular immunity. This causes inflammation in immunosenescence to be non-specific inflammation and when given a stimulus (the presence of a pathogen or in this case a vaccine), the resulting inflammation, as an immune response, is inadequate [48]. However, different from the theory, sCD28 is not correlated with the decline of neopterin serum, unlike sCD80. Some studies mentioned that CD28 downregulation occurs during both T-cell senescence and differentiation into terminal effector cells, thus it lacks the specificity to stand alone as a senescence marker [49]. Consequently, evaluating immunosenescence need combination with other cellular markers. However, sCD80 is probably more specific as a T cell senescence biomarker but needs further study to be confirmed.

A study by Wagner et al found a decrease in cellular and humoral immune responses in geriatrics following vaccine administration [50]. Another study by Xiao et al. reported a decrease in the cellular immune response following vaccine administration with an inactivated SARS-CoV-2 vaccine in geriatrics. In addition, it was also reported that geriatrics have a slower immune response to form following vaccine administration [51]. Different from previous research, this study conducted a cohort study that measured the decline in cellular immune response, namely neopterin levels, and the decline in neopterin levels correlated with markers of T cell aging.

5. Study Limitations

Several limitations of this study should be acknowledged. First, the absence of an internal, contemporaneously measured young-adult control group limits our ability to make definitive comparisons. Our characterization of the older adult cohort relies on reference values derived from three external studies, making this historical comparison inherently vulnerable to inter-assay variation across different laboratory and enzyme-linked immunosorbent assay (ELISA) platforms. Consequently, we cannot categorically conclude that all older subjects are immunosenescent; these findings must instead be interpreted cautiously as indicative of age-related immunological trends. Second, neopterin levels were evaluated exclusively at weeks 6 and 24 post-vaccination, which restricts the temporal resolution of the data. Third, this study did not compare the decline in post-vaccination immune responses between cohorts with and without T-cell senescence, and our analysis was restricted to a single marker of cellular immunity rather than a direct assessment of actual T-cell functional activity. Furthermore, potential lifestyle and physiological confounding variables—such as stress, sleep disturbances, intestinal dysbiosis, physical activity, smoking status, alcohol consumption, dietary habits, vitamin C and D supplementation, albumin levels, and heterogeneous comorbidities—were not fully controlled. Demographically, as a single-center study, the generalizability of these findings is limited and may not fully represent the broader landscape in Indonesia, where the majority of the older adult population has now received third or fourth booster doses using mRNA platforms. Furthermore, an a priori sample size calculation was not performed due to the exploratory nature of this study. A post-hoc power analysis conducted for the primary correlation with the final sample size and a two-tailed alpha of 0.05 yielded a statistical power of approximately 68%. This lower power threshold necessitates caution, as weak-to-moderate correlations may be more susceptible to sample size constraints. Consequently, future multi-center investigations incorporating a parallel young control group analyzed with identical assay kits are warranted to comprehensively validate these observations and elucidate the kinetics of cellular immunity following vaccination.

6. Conclusions

An elevation in sCD28, sCD80, and sCTLA-4 was observed across the older adult cohort and positively correlated with serum neopterin levels, suggesting that T-cell senescence drives inflammaging. Furthermore, an increase in these soluble biomarkers correlated with a more rapid decline in serum neopterin concentrations over the 18-week interval between weeks 6 and 24 following the second dose of the CoronaVac vaccine. Taken together, these findings indicate that older adults exhibit features of T-cell senescence and concurrent inflammaging, both of which are associated with an accelerated post-vaccination decline in neopterin levels. However, further investigations remain necessary to directly evaluate the underlying functional activity of the cellular immune response.

Author Contributions

Conceptualization, A.A. and K.H.; methodology, A.A., K.H., F.S., F.M.S., and A.S.; software, A.A. and D.P.S.; validation, A.A., K.H., H.S., and A.S.; formal analysis, A.A. and K.H.; investigation, A.A., D.P.S., F.S., and F.M.S.; resources, A.A. and K.H.; data curation, A.A., K.H., H.S., and A.S.; writing—original draft preparation, A.A. and D.P.S.; writing—review and editing, A.A., K.H., D.P.S., H.S., F.S., and A.S.; visualization, A.A., D.P.S., and F.M.S.; supervision, K.H., H.S., and A.S.; project administration, A.A. and K.H.; funding acquisition, K.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

All authors have done work in the field of human and animal also have observed the principles of professional ethics and human and animal rights. The Ethics Commission of Universitas Brawijaya Malang, Indonesia, with regard to the protection of human rights and welfare in medical research, has carefully reviewed this research and approved it with the ethical approval Number: 134/EC/KEPK – S3/06/2023 on June 21, 2023.

Informed Consent Statement

Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets generated and/or analyzed during this study are not publicly available due to institutional regulations but may be obtained from the corresponding author upon reasonable requests and with appropriate institutional approvals.

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Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

During the preparation of this work, the authors used online translator (google translate) for quick translate. However, the authors subsequently reviewed and edited the content as necessary and take full responsibility for the final content of the published article.

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